SYNTHESIS, CHARACTERIZATION AND EVALUATION OF NOVEL ISATIN ANALOGUES

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Abstract

The present study is aimed to carry out the synthesis of Benzylimino- isatin *Mannich* base derivatives. For newer derivatives, Benzylimino-isatin as lead molecule by combining several secondary amines followed by formaldehyde will be synthesized as per literature method. Then, structures will be assigned by FT-IR and ^{1}H NMR analysis. Further, the compounds are evaluated for biological activities such as anti-cancer and anti-microbial activities. Our current research work deals with manually designed, library of compounds (IM1-20) bearing benzylimino-isatin scaffold that performed docking study with *E.coli Quinol-Fumarate Reductase* with Bound Inhibitor HQNO enzyme (1kf6) [PDB code 1kf6] using Molegro Virtual Docker Evaluation version (MVD 2013.6.0) the best compounds were selected based on their Moldock score in order to synthesis of benzylimino-isatin (IS1)([(3Z)- 3-(benzylimino)-1,3-dihydro-2H-indol-2-one)]) and benzylimino-isatin mannich base derivatives with isatin as a parent moiety.

Key words: Benzylimino- isatin, FT-IR, NMR

Introduction

A pure chemical substance composed of more than one element is called as compound. Drug design, often referred to as rational drug design or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target. Drug design frequently but not necessarily relies on computer modeling techniques. This type of modeling is sometimes referred to as computer-aided drug design. In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

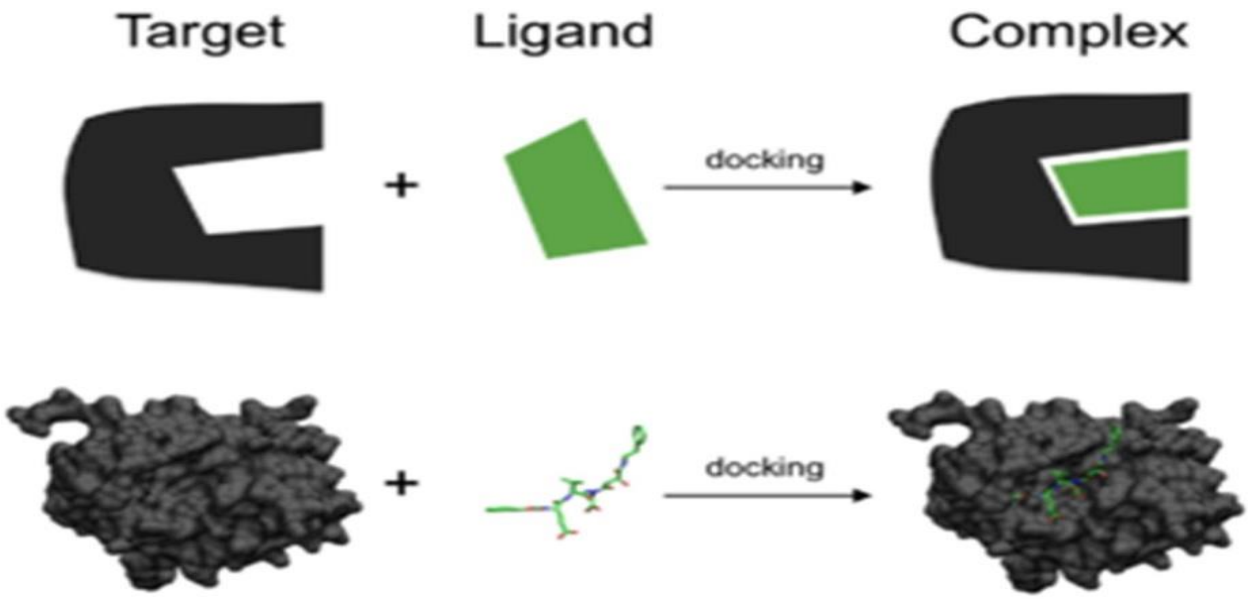


FIG.1: SCHEMATIC ILLUSTRATION OF DOCKING A SMALL MOLECULE LIGAND (GREEN) TO A PROTEIN TARGET (BLACK) PRODUCING A STABLE COMPLEX.

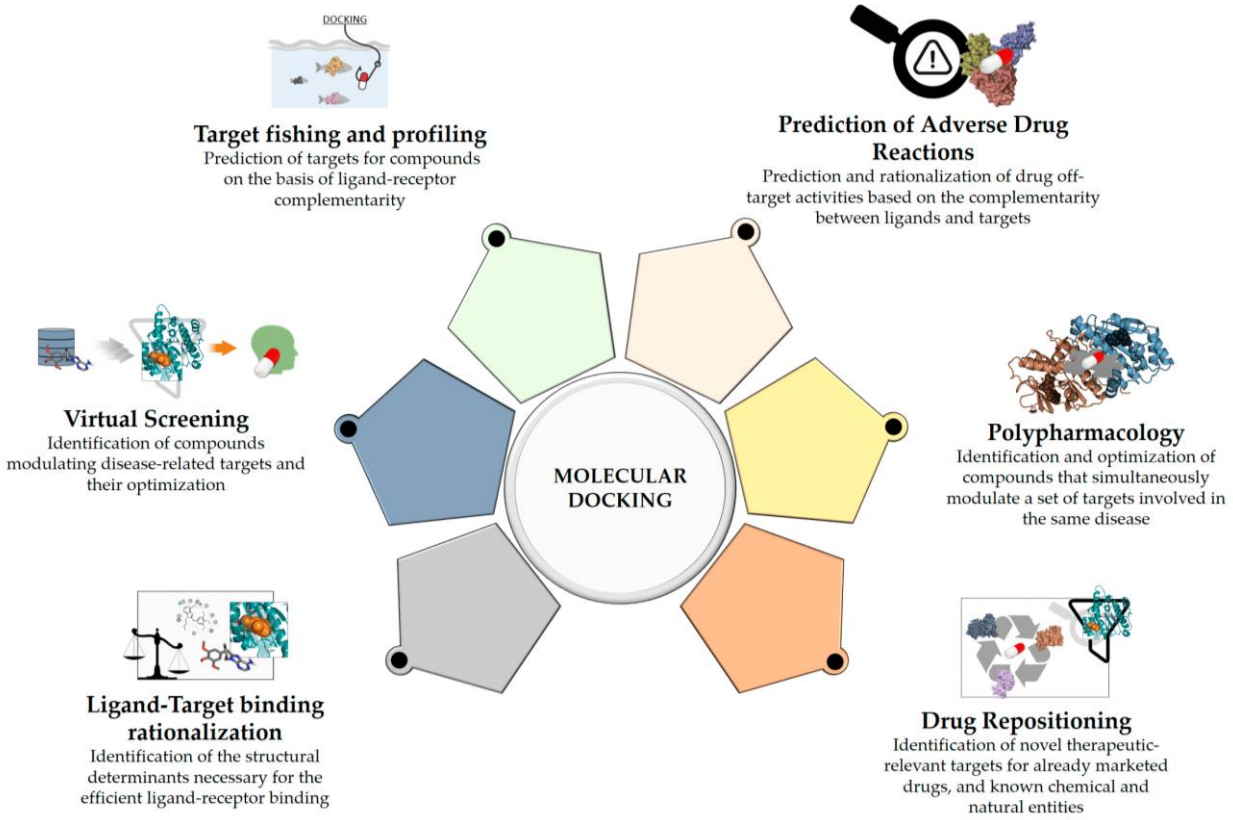


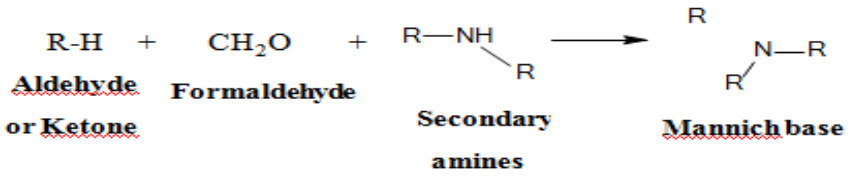
FIG.2: APPLICATIONS OF MOLECULAR DOCKING

ISATIN

Isatin(indoline-2,3-dione), is an indole derivatives (Ex: Indozoline, Fluvaisatin), possessing an indole nucleus with two chemically distinct cyclic carbonyl groups, keto and lactam.

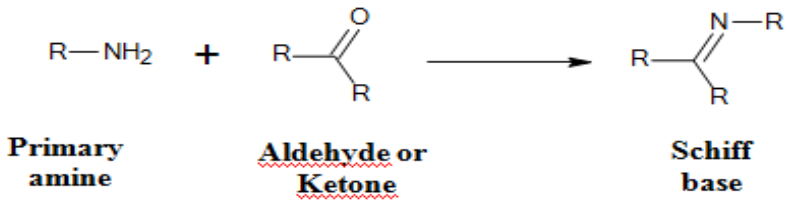
MANNICH BASE

A Mannich base is a beta-amino-ketone, is an end product in the Mannich reaction, is the condensation reaction in which the compound containing active hydrogen atom is allowed to react with formaldehyde and an NH-amine derivative.



SCHIFF BASE

A Schiff base is a nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by C=N-R group. It is usually formed by condensation of an aldehyde or ketone with a primary amine.



The present study is aimed to carry out the synthesis of Benzylimino- isatin *Mannich* base derivatives. For newer derivatives, Benzylimino-isatin as lead molecule by combining several secondary amines followed by formaldehyde will be synthesized as per literature method. Then, structures will be assigned by FT-IR and ^{1}H NMR analysis. Further, the compounds are evaluated for biological activities such as anti-cancer and anti-microbial activities.

Materials and methods

TABLE 1: Company name of the chemicals used in synthesis

S.NO	CHEMICALS	COMPANY NAME
1.	Isatin	Sisco Research Laboratory
2.	Benzylamine	High Purity Laboratory Chemicals
3.	Glacial acetic acid	Fisher Scientific
4.	Dimethlyamine	Loba Chemie
5.	Piperazine	HiMedia Laboratories
6.	Pthalimide	Santai Labs
7.	Diphenylamine	Sisco Research Laboratory
8.	1-Methly piperazine	Spectrochem
9.	Morpholine	Spectrochem

10	Formaldehyde	Sai Chemicals
11.	Ethanol	Sisco Research Laboratory
12.	Methanol	Molychem
13.	Chloroform	Sisco Research Laboratory
14.	DMSO	Fischer inorganics & aromatics Ltd
15.	Ethyl acetate	Chem India Petrochems
16.	Hexane	Roshan Chemical Industry
17.	Benzene	Alpha Chemika
18.	Pet. ether	Lab-Chem Corporation
19.	Silica gel G	Thomas baker

Software used in docking study

The docking study was performed using Molegro Virtual Docker Evaluation Version (MVD 2013.6.0), which focused on molecular docking simulations.

Table 2: Instruments and its model

S.NO	INSTRUMENT	MODEL
1.	Digital balance	ELB 300 SHIMADZU
2.	Magnetic stirrer	MCS 66
3.	Rota vaccum evaporater	RVO 400
4.	Melting point apparatus	M-565
5.	FT-IR Spectrophotometer	IRTRACER-100 SHIMADZU
6.	1H-NMR Spectrometer	SHIMADZU-400
7.	MTT Assay reader	Bio-Rad-680

Results and discussion

Table. 3: Selected Docking Scores of compounds in the cavity 1 of 1kf6 Enzyme

Com. Code	Mol-dock score	Re-rank score	H-bond score
IS1	-136.065	-104.478	-3.179
IM1	-146.225	-105.988	-3.693
IM3	-183.245	-140.876	-1.402
IM4	-167.199	-123.731	-3.887
IM5	-177.219	-129.985	-2.241
IM19	-186.228	-135.796	-1.511
IM20	-143.335	-114.225	-2.298

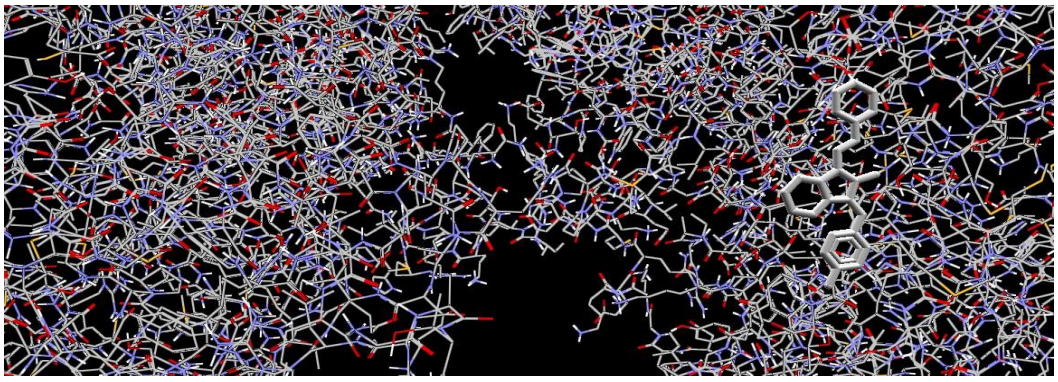
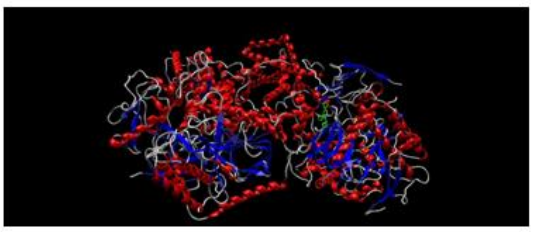


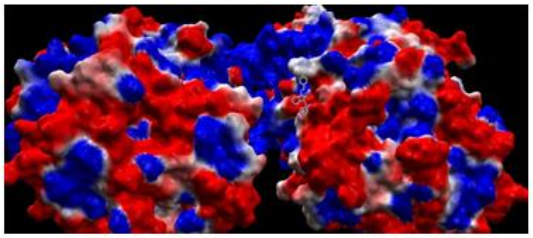
Fig-3 : DOCKING VIEW OF LIGAND (IM19) AND PROTEIN (1KF6)

Docking studies of different benzylimino-isatin derivatives (IM1, IM2, IM3, IM4, IM5, IM6, IM7, IM8, IM9, IM10, IM11, IM12, IM13, IM14, IM15, IM16, IM17, IM18, IM19 & IM20) were performed successfully inside the highest volume cavity measure the affinity were Mol Dock, re-rank and H- bond score of the above designed compounds with *E. coli* Quinol-Fumarate Reductase with Bound Inhibitor HQNO enzymes (1kf6). It facilitated us to identify relevant H-bond interaction (via H-bond score) that occurs between each ligand and the amino acid residues of the active site of enzyme in order to obtain conformations achieved with these molecules. Although the key moieties of all the compounds were similar but each individual compound showed interaction up to a variable extent.

The best Docking Score of the compounds (table 5) were selected for the synthesis as well as biological evaluation, among various compounds, IM19 ([(3Z)-3-(benzylimino)-1-[(morpholin-4-ylmethyl)-1,3-dihydro-2H- indol-2-one]) showed highest MolDock score (-186.228) as well as re-rank score (-135.796) as compared to the other benzylimino-isatin derivatives. While compound IM16 showed poor MolDock score (-84.453) as well as re-rank score (-71.564) as compared with other Mannich bases of benzylimino- isatin derivatives. Docking view, hydrophobic and steric interactions and secondary view of IM19 ([(3Z)-3-(benzylimino)-1-[(morpholin-4-ylmethyl)- 1,3-dihydro-2H-indol-2-one]) with 1kf6 enzyme have been shown in Figs. (15, 16 & 17) respectively.



SECONDARY VIEW OF COMPOUND IM19 (GREEN COLOUR) WITH 1KF6 ENZYME HAVING PDB ID: 1KF6 (RED AS A-HELICES AND BLUE AS B-SHEETS)



HYDROPHOBIC INTERACTIONS OF COMPOUND IM19 WITH 1KF6 ENZYME SHOWING HYDROPHOBIC AND HYDROPHILIC SURFACES

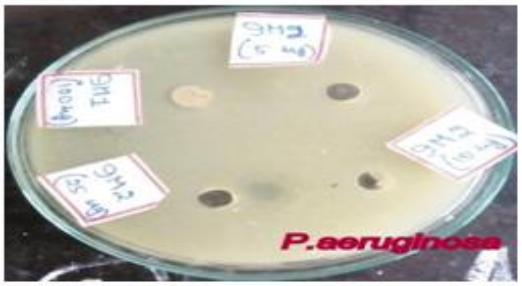
TABLE.4: Antimicrobial activity of the synthesized compounds by disc diffusion method

S. No	Com. Code	Zone of inhibition (mm)											
		S. aureus			*B. subtilis*			*P.aeruginosa*			*E. Coli*		
		25	50	100	25	50	100	25	50	100	25	50	100
1.	IS1	9	9	9	9	10	9	10	12	14	9	10	10
2.	IM1	9	11	13	10	10	14	10	12	13	9	9	11
3.	IM3	10	10	12	10	11	11	10	14	19	9	10	11
4.	IM4	9	9	9	11	14	14	11	12	19	9	9	11
5.	IM5	9	9	10	9	10	14	10	12	14	9	10	10
6.	IM19	11	11	12	10	14	12	9	10	15	9	9	11
7.	IM20	9	9	9	9	9	9	10	15	19	9	10	11
8.	Ciprofloxacin	20 (10 µg/ml)			30 (10 µg/ml)			30 (10 µg/ml)			20 (10 µg/ml)		

TABLE.5: Antimicrobial activity of the synthesized compounds by well diffusion method

S.No	Com. Code	Zone of inhibition (mm)											
		S. aureus			*B. subtilis*			*P.aeruginosa*			*E. Coli*		
		25	**50**	**100**	**25**	**50**	**100**	**25**	**50**	**100**	**25**	**50**	**100**
1.	IS1	10	10	10	10	11	15	11	13	15	10	11	11

2.	IM1	12	12	13	11	15	10	10	20	20	10	10	12
3.	IM3	11	11	13	11	11	12	10	10	20	10	11	12
4.	IM4	10	10	10	12	15	15	12	13	20	10	10	12
5.	IM5	10	10	10	10	11	15	11	13	15	10	11	11
6.	IM19	12	12	13	11	15	13	10	20	20	10	10	12
7.	IM20	10	10	10	10	10	10	10	10	20	10	11	11
8.	Ciprofloxacin	20 (10 µg/ml)			30 (10 µg/ml)			30 (10 µg/ml)			20 (10 µg/ml)		



IM1

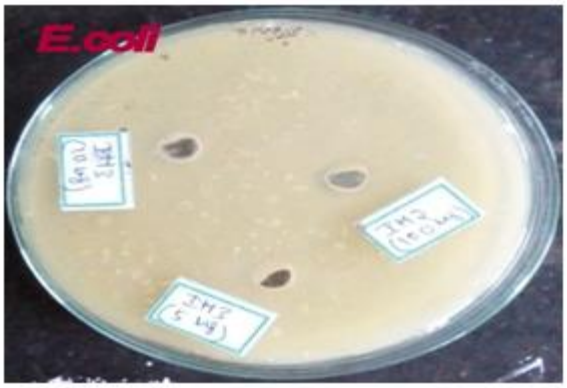
IM2

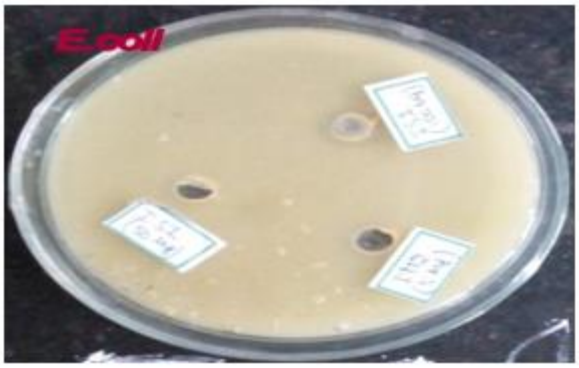
ZONE OF ANTI-MICROBIAL INHIBITION (10, 25, 50 & 100 µG/0.1 ML)

AGAINST *P.AERUGINOSA*

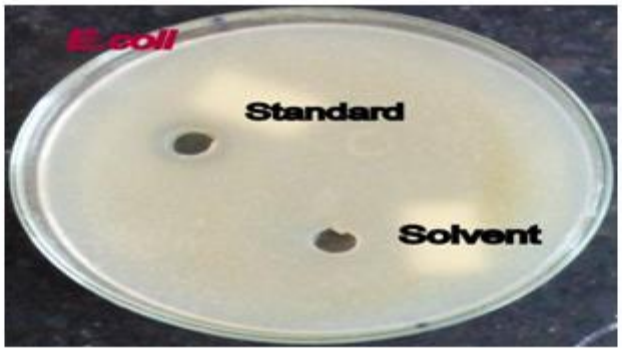
IM3

IM4

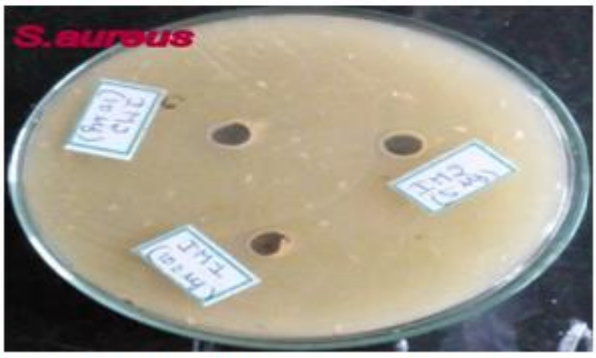




ZONE OF ANTI-MICROBIAL INHIBITION OF IM2 (10, 25, 50 & 100 µG/0.1 ML) AGAINST *E.COLI*



ZONE OF ANTI-MICROBIAL INHIBITION OF IM2 (10, 25, 50 & 100 µG/0.1 ML) AGAINST B. SUBTILIS



ZONE OF ANTI-MICROBIAL INHIBITION OF IM2 (10, 25, 50 & 100 µG/0.1 ML) AGAINST S. AUREUS

ANTICANCER ACTIVITY

The anticancer activity for synthesized compounds was performed in HeLa cell line by MTT assay method. The results of the synthesized compounds in the form of IC50 was posted. The compound IM3 [(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3- dihydro-2H-indol-2-one] and IM20 2-{[(3Z)-3-(benzylimino)2-oxo-2,3- dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3(2H)-dione has good activity about 0.327, 0.392 µg/ml respectively while other compounds shows moderate activity. 5FU used as standard and it have IC50 about 0.21 µg/ml.

Conclusion

Our current research work deals with manually designed, library of compounds (IM1-20) bearing benzylimino-isatin scaffold that performed docking study with *E.coli Quinol-Fumarate Reductase* with Bound Inhibitor HQNO enzyme (1kf6) [PDB code 1kf6] using Molegro Virtual Docker Evaluation version (MVD 2013.6.0) the best compounds were selected based on their Moldock score in order to synthesis of benzylimino-isatin (IS1)([(3Z)-3-(benzylimino)-1,3-dihydro-2H-indol-2-one)]) and benzylimino-isatin mannich base derivatives with isatin as a parent moiety.

Benzylimino-isatin (IS1) ([(3Z)- 3-(benzylimino)-1,3-dihydro-2H-indol-2-one)]) and benzylimino-isatin mannich bases such as IM1 [(3Z)-3-(benzylimino)-1-[(dimethylamino) methyl]-1, 3- dihydro-2H-indol-2-one], IM3 [(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one], IM4 [(3Z)-3-(benzylimino)-1-[(piperazin- 1-yl methyl)-1, 3-dihydro-2H-indol-2-one], IM5 [(3Z)-3-(benzylimino)-1-[(4- methylpiperazin-1-yl) methyl]-1,3-dihydro-2H-indol-2-one], IM19 [(3Z)-3- (benzylimino)-1-[(morpholin-4-yl methyl)-1,3-dihydro-2H-indol-2-one] & IM20 [2-{[(3Z)-3-(benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole- 1,3(2H)-dione] were synthesized by the suitable experimental procedure.

The synthesized compounds were screened for *in-vitro* anti-microbial activity by disc diffusion method as well as well diffusion method and *in-vitro* anti-cancer activity by MTT assay method. Among the evaluated compound, three compounds such as IM3 [(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one], IM4 [(3Z)-3-(benzylimino)-1-[(piperazin- 1-yl methyl)-1, 3-dihydro-2H-indol-2-one] & IM20 [2-{[(3Z)-3-(benzylimino)2- oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3(2H)-dione] have good *in-vitro* anti-microbial activity at a dose of 50 and 100 µg/0.1 mL, when compared to standard drug Ciprofloxacin at a dose of 10 µg/0.1 mL.Among the evaluated compounds, two compounds such as IM3 [(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one] & IM20 2-{[(3Z)-3- (benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3(2H)- dione have good *in-vitro* anti-cancer activity with IC50 0.392 µg/mL and 0.327 µg/mL aagainst human cervical cancer cell line (HeLa cell line) when compared to standard about 5FU with IC50 0.21 µg/mL.

From the above facts it can be suggested that the benzylimino-isatin mannich base derivatives finds an interesting field of research because of their varied pharmacological activities.

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