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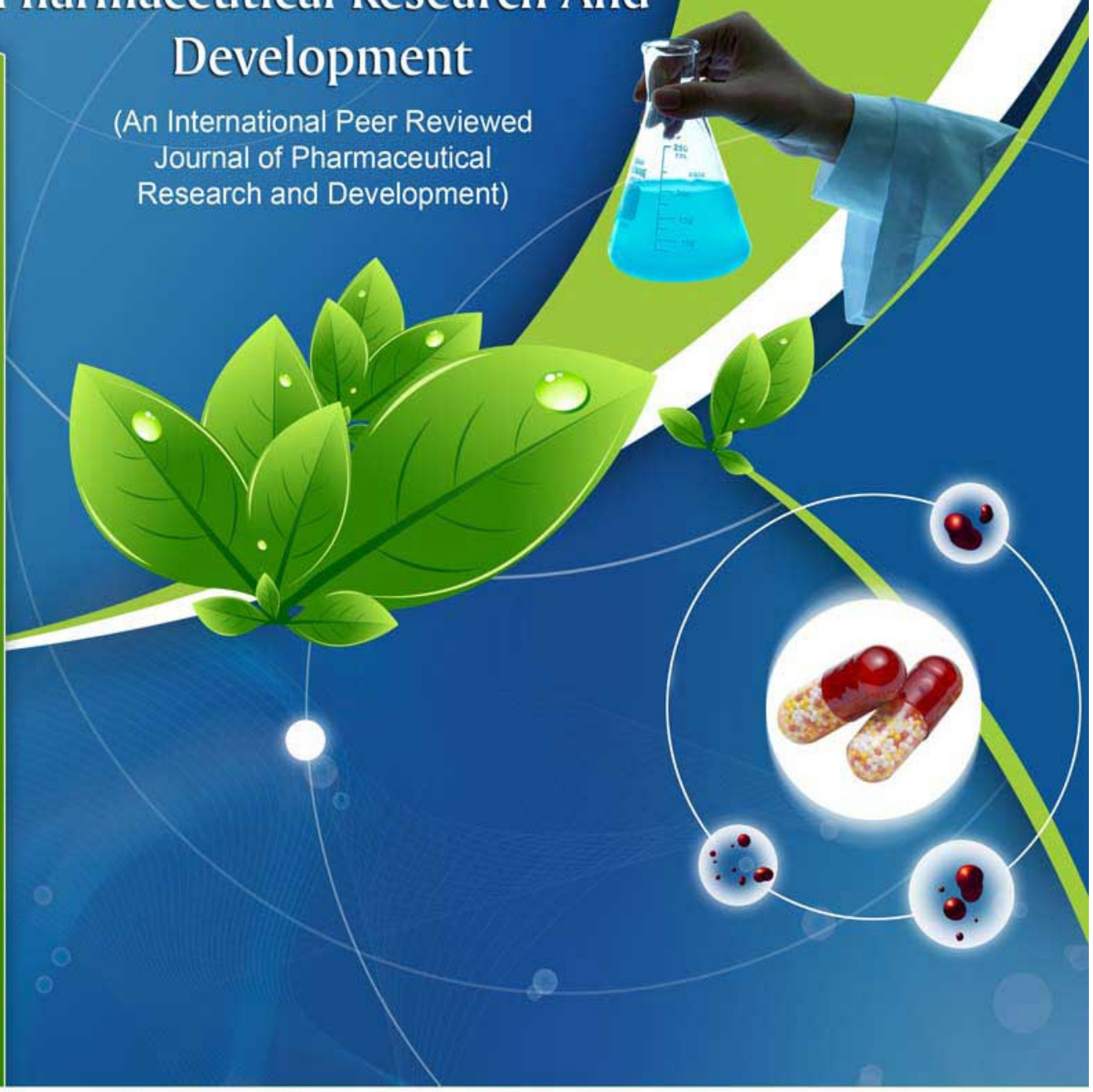

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Research Article

ANTIMICROBIAL ACTIVITY OF SCHIFF BASE OF OFLOXACIN

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ABSTRACT

The Antimicrobial activity of Schiff base of ofloxacin, was investigated in vitro under aseptic conditions, using the disk diffusion method, against various gram positive and gram negative pathogenic microorganisms such as Pseudomonas aeruginosa (P.A.), Staphylococcus aureus (S.aureus), Helicobacter pylori (H. pylori), Escherichia coli (E. coli), Methicillin-resistant Staphylococcus aureus (MRSA) and some fungal strains such as, Aspergillus fumigatus, Pneumocystis carinii and Aspergillus niger. A series of these compounds were prepared and have been shown to inhibit pathogenic growth, judging from the area of the zone of inhibition. The area of zone of inhibition of compounds found from 6 mm^2 to 48 mm^2. Among the synthesized compounds; ***Compound SV-14*** *(6-[(4,7-Dimethyl-benzothiazole-2-carbothioyl)-hydrazono]-8-fluoro-3-methyl-9-(4-methyl-piperazin-1-yl)-2,3-dihydro-6H-1-oxa-3a-aza-phenalene-5- carboxylic acid, showed good activity against P.A. (zone of inhibition 8 mm^2 at 30 µg/ml), H. pylori (zone of inhibition 6 mm^2 at 30 µg/ml) and E. coli (zone of inhibition 8 mm^2 at 30 µg/ml); Compounds* ***SV-8, SV-9, SV-10, SV-11, SV-12, SV-13 and SV-14*** *exhibited promising antibacterial activity. The target compounds showed in vitro antibacterial & antifungal activity less than reference antibiotic ofloxacin.*

Keywords: *Antimicrobial, Schiff base, Zone of Inhibition, Ofloxacin.*

INTRODUCTION

Quinolones are synthetic antibacterial compounds based on a 4- quinolone skeleton. Quinolones have been clinically successful and more used to treat bacterial infection. Fluoroquinolones target bacterial type-II topoisomerases, generally DNA gyrase in gram negative bacteria and DNA topoisomerase in gram positive bacteria [1-3]. The synthesis and evaluation of over 10,000 quinolone derivatives resulted in thorough knowledge of the structure-activity relationship for many quinolone substituents [4].

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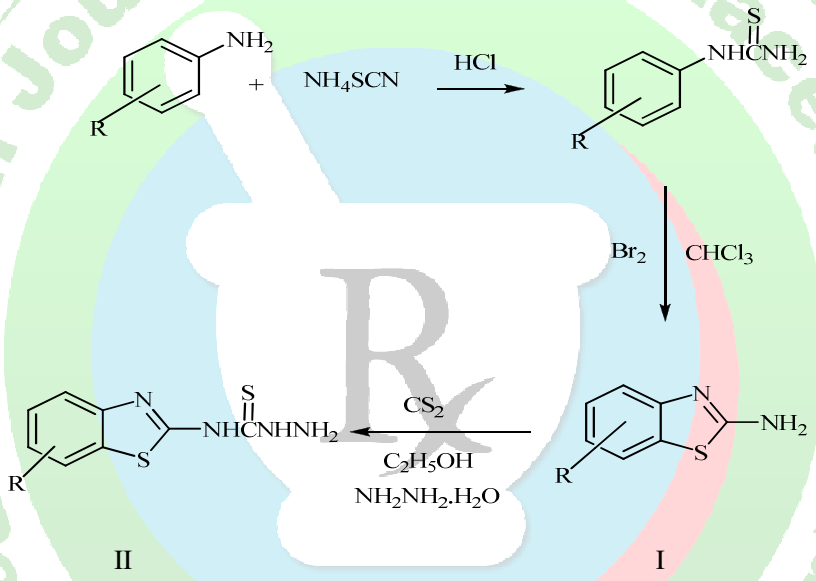
The first analogue of this class, nalidixic acid, was synthesized in 1962 [5] and used for the treatment of urinary tract infections [6]. It is more active against Gram-positive than Gram-negative organisms [7].

Fluoroquinolones are extremely useful for the treatment of a variety of infections, including urinary tract infections, soft tissue infections, respiratory infections, bone-joint infections, typhoid fever, sexual transmitted diseases, prostatitis, community acquired pneumonia, acute bronchitis and sinusitis [8-9]. Recently a relatively new approach to the rational design of antimicrobial agents has been introduced based on some new quinolone molecules [10]. Ciprofloxacin, [1- cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl)-3-quinoline carboxylic acid], a typical second generation fluroquinolone, has been in clinical use for

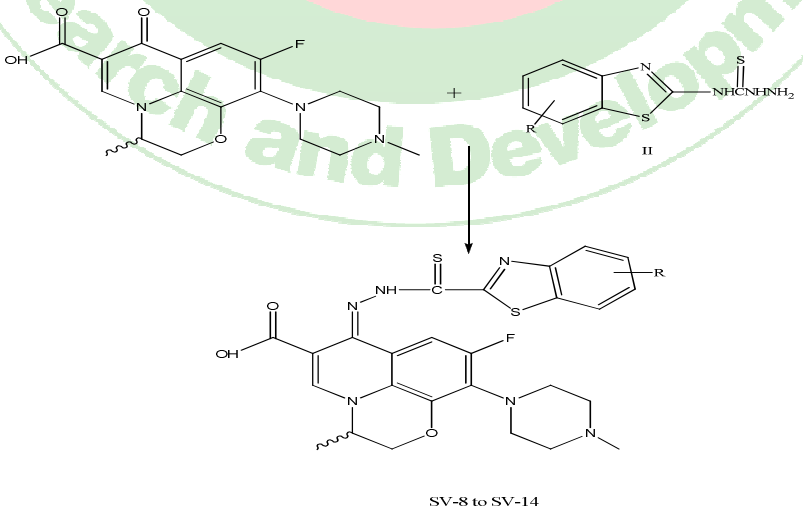
more than a decade and sold for $US 1.5 billion in 1996 [11]. The drug has been the center of great interest and success, and more Different structural modifications in the quinoline nucleus have been made to increase antimicrobial activity and improve its performance. During 1980's, it was discovered that a fluorine atom at position 6 and piperazine ring at position 7 greatly enhance the spectrum of activity of these antibiotics [13, 14]. In a structure activity relationship 4-oxo group is considered essential for antibacterial activity and therefore, modifications of this moiety has been not much explored. In the present study the modification of 4-oxo group has been explored to confirm whether this group is really essential or not. On the other hand 2- amino benzthiazole derivatives have shown promising antibacterial activities. Therefore, shiff bases of 2- amino benzthiazole with 4-oxo group of fluoroquinolones are expected to enhance antibacterial activity of fluoroquinolones. These compounds were prepared as per scheme given below.

than 15000 articles have been published about it [12].

Step 1:-



Step 2:-



Scheme: Synthesis of Schiff base of ofloxacin

MATERIAL AND METHODS

Melting point of the synthesized compounds were determined by open capillary method. The purity of the compounds was checked using TLC Plates, using chloroform**:** methanol (8:2) solvent system. The developed chromatographic plates were visualized in saturated Iodine chamber. IR Spectra were recorded using KBr on BRUKER Spectrophotometer, NMR spectra in $CDCl_3$ on FT-NMR instrument using TMS as internal standard.

GENERAL PROCEDURE FOR SYNTHESIS OF SCHIFF BASES

Compound I: Benzothiazole-2-yl amine

Compound [I] was synthesized by heating aniline (0.3 moles) and concentrated hydrochloric acid (25ml). 0.4 mole of saturated solution of ammonium thiocynate in water (30gm in 60ml water) was added slowly in above solution. The reaction mixture was boiled until the solution got turbid. The solution was poured in ice water. The precipitate was filtered and recrystallized from ethanol to give phenylthiourea. Phenylthiourea (0.1 mole) in glacial acetic acid (75ml) was brominated by using bromine solution in glacial acetic acid (5%) till the orange yellow color appeared. The slurry was poured in cold water and make alkaline with 50% aq. Ammonia solution. The precipitate was filtered and washed with water, dried and recrystallize by using ethanol. The melting point was found to be 156°C [15].

Compound II: N-(benzothiazol-2-yl) hydrazine carbothioamide

0.01mole of product I was dissolved in ethanol using potassium hydroxide as base. An equi-molar amount of CS_2 and hydrazine hydrate were then separately added drop wise to the solution of product I with stirring at 0-5°C temperature. A light yellow solid was precipitated at the end of the reaction. The product obtained was filtered and recrystallized with ethanol.

Schiff base of Ofloxacin with N-(benzothiazol-2-yl) hydrazine carbothioamide

Equimolar quantities (approx. 0.01mole) of Compound II and Ofloxacin were separately dissolved in a minimum amount of ethanol and then they were mixed together followed by addition of 5 ml glacial acetic acid .The solution was refluxed for 10 hrs. Then cooled to room temperature and poured into ice cold water .The solid product was collected through filtration and then were air dried. The product was re-dissolved in ethanol for re-crystallization and filtered to give a product. The physicochemical properties of the Schiff bases are described in Table 1.

ANTIMICROBIAL ACTIVITY

Preparation of Suspension of Bacteria

2 ml normal saline (0.85% w/v) was taken in test tubes and then plugged with cotton, raped with news paper with help of cello tape. Test tubes were put in autoclave for sterilization for 15 lbs for 20 min. After autoclaving take 1-2 colonies of bacteria from sub cultured bacterial plate with the help of loop. Colonies were dissolved in normal saline with rub on side of test tube with stirring. Turbidity of tubes were marked and add more colonies if needed.

Procedure for Sensitivity Test

Preparation of Muller Hinton Agar Plates

3.0 mg Muller Hinton agar media was dissolved properly in 80 mL Distilled water in 250 mL conical flask with stirring (For preparation of four plates).The mouth of conical flask was plugged with cotton, rapped with news paper with help of cello tape. Conical flask was put in autoclave for sterilization for 15 lbs for 20 min. After autoclaving, warm 20-25 mL media was poured on petri dish per plate in front of laminar flow. The media was left until solidified in petri disk. After that plate was put in incubators for drying the water vapour in plate. Now agar plate was ready for use.

Preparation of Compounds Solution

5 mg compound was dissolved in 1 ml DMSO: PEG=1:10 in test tube with vertex stirring, heated if required (conc. of DMSO was not increased above 1%). Each tube was given a code number. The prepared plate was divided into four quadrants with the help of marker. The same code was given to each quadrant as given to code to test tube containing solution of compound. One plate was swab from one bacterial suspension with the help of cotton swab, coded the name of bacteria on each plate.

With the help of micro pipette, 10-20 µl solution of compound was dropped on same code of quadrant as given on the test tube containing solution of compound. All plates were put in incubator for incubation for 18-24 hrs. After18-24 hrs, the plates were viewed. If the specific compound was sensitive for specific bacteria, then growth was found in whole plate except where solution of compound was dropped. If the specific compound was not sensitive for specific bacteria, then growth was found in whole plate including where solution of compound was dropped.

Reference and Control

The references were antibiotic in nature. *Ofloxacin* was chosen as the reference for all bacterial species. *Nystatin* was used as the reference for the fungus. The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion as reported [17].

Aseptic conditions

The aseptic chamber consists of a wooden box (1m x 1m x 0.5m) with a door which was cleaned with 70% ethanol and irradiated with short wave UV light for 1 hour.

Mother plates

These were made by culturing all bacterial strains on PDA (Potato dextrose Agar). A sterilized 9 cm cork borer was used to cut agar discs in the plate.

Potato dextrose agar (PDA)

This is an agar medium on which the fungi were cultured. The potato was peeled and 200g weighed, finely chopped and boiled to a mash in distilled water. The dextrose was weighed (12.5g) and placed in a 1L measuring cylinder. Agar was weighed (12.5g) and added to the measuring cylinder (with the dextrose).The potato mash was stirred and strained into the cylinder. Distilled water was added to make up the solution to 500mL.The contents was continuously stirred until consistency was achieved and was then poured into a conical flask, plugged with cotton wool, over which aluminum foil was tightly wrapped. The flask was then autoclaved at 121°C, 15psi for fifteen minutes [17].

Agar diffusion Technique

The spore suspension of pathogens was seeded into a molten PDA medium or poured into petri plates. When the medium solidified, a 9 cm well was made at the centre of the plate with the help of a sterile 9 cm cork borer. A solution of the test compound (1), at a concentration of 1mg in 1ml was transferred into the well and incubated for three days. The zone of inhibition in mm2 was measured for the test compound and recorded. From these values, the area of inhibition was calculated [18].

RESULT AND DISCUSSION

Antibacterial Activity

The *in vitro* antibacterial activity of Schiff bases of Ofloxacin was investigated against gram positive organisms (*Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus)* and gram negative organisms *(Helicobacter pylori, Escherichia coli* and *Pseudomonas aeruginosa).* Results are summarized in Table 2 along with standard drug.

Table 1: Physicochemical properties of the Schiff bases (SV-8 to SV-14)

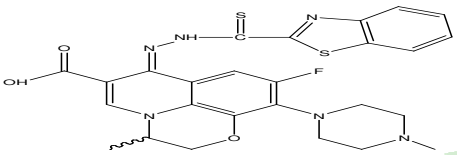
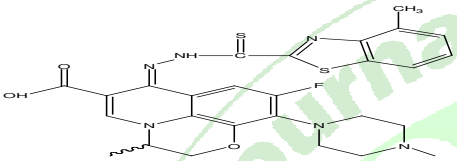
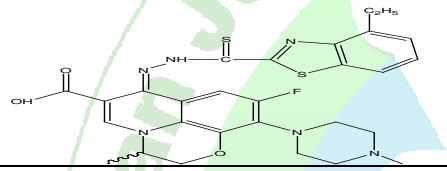
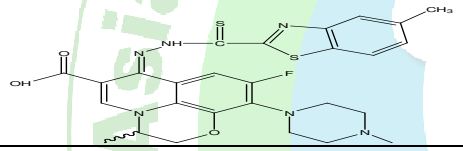
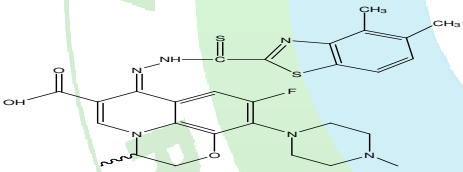
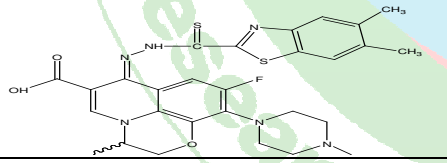
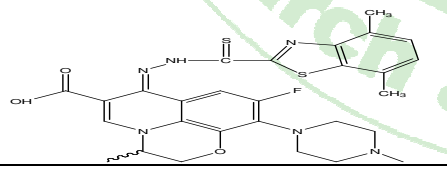
S. No.	Code	Compound Structure	Mol. Formula	Mol. Weight	Melting Point	% Yield	Solubility	Elemental Analysis (%) Calculated/Found		
								C	H	N
8.	SV-8		$C_{25}H_{23}FN_6O_3S_2$	538.13	255°C	48%	Ethanol	55.75 55.72	4.30 4.32	15.58 15.60
9.	SV-9		$C_{26}H_{25}FN_6O_3S_2$	552.14	271°C	38%	Ethanol	56.51 56.53	4.56 4.58	15.21 15.24
10.	SV-10		$C_{27}H_{27}FN_6O_3S_2$	566.16	236°C	50%	Ethanol	57.23 57.24	4.80 4.82	14.83 14.86
11.	SV-11		$C_{26}H_{25}FN_6O_3S_2$	552.14	198°C	49%	Ethanol	56.51 56.53	4.56 4.58	15.21 15.24
12.	SV-12		$C_{27}H_{27}FN_6O_3S_2$	566.16	212°C	30%	Ethanol	57.23 57.25	4.80 4.82	14.83 14.86
13.	SV-13		$C_{27}H_{27}FN_6O_3S_2$	566.16	230°C	49%	Ethanol	57.23 57.25	4.80 4.82	14.83 14.86
14.	SV-14		$C_{27}H_{27}FN_6O_3S_2$	566.16	276°C	46%	Ethanol	57.23 57.25	4.80 4.82	14.83 14.86

Table 2: Antimicrobial Activity of Synthesized compound (Ofloxacin derivatives)

S. N.	Compound Code	Conc. (µg/ml)	*P. aeruginosa*		*H. pylori*		*E. coli*		*S. aureus*		*M.R.S.aureus*	
			ZOI	MIC (µg/ml)	ZOI	MIC (µg/ml)	ZOI	MIC (µg/ml)	ZOI	MIC (µg/ml)	ZOI	MIC (µg/ml)
1.	SV-8	300	35	66	22	100	34	61	14	133	40	30
		100	12		8		13		6		18	
		30	0		0		0		0		8	
2.	SV-9	300	30	80	15	133	32	66	12	133	38	40
		100	10		6		12		6		15	
		30	0		0		0		0		6	
3.	SV-10	300	18	133	28	80	24	100	10	240	25	88
		100	6		10		8		0		9	
		30	0		0		0		0		0	
4.	SV-11	300	18	133	22	100	26	133	19	114	35	40
		100	6		8		6		7		14	
		30	0		0		0		0		6	
5.	SV-12	300	25	100	18	133	20	133	22	100	35	40
		100	8		6		6		8		12	
		30	0		0		0		0		6	
6.	SV-13	300	20	100	18	133	38	40	18	133	28	66
		100	8		6		14		6		12	
		30	0		0		6		0		0	
7.	SV-14	300	35	30	36	40	45	30	16	133	25	80
		100	15		16		22		6		10	
		30	8		6		8		0		0	
8.	Ofloxacin (Control)			0.41		0.69		0.31		0.78		0.19

PA= *Pseudomonas aeruginosa*, SA= *Staphylococcus aureus*, H. Pylori= *Helicobacter pylori,* E.Coli= *Escherichia coli,* MRSA= *Methicillin-resistant Staphylococcus aureus,* ZOI= Zone of Inhibition, Conc.= Concentration

Table 3: Antifungal Activity of Synthesized compound

Compound Code	*Aspergillus niger (AN)*	*Pneumocystis carinii (PC)*	*Aspergillus fumigatus (AF)*
SV-8	12	10	0
SV-9	0	0	0
SV-10	8	0	0
SV-11	0	0	0
SV-12	0	0	0
SV-13	0	0	0
SV-14	12	18	14

All analogues, showed comparable antibacterial activity at the dose 300 µg/ml against all the tested strains. Results indicate that compound SV-14 showed maximum activity against *H. pylori* (zone of inhibition=36 mm^2 and MIC=40 µg/ml at the dose of 300 µg/ml) in comparison to other strains used by us. Compounds SV-8, and SV-14 showed maximum activity against *P. aeruginosa* (zone of inhibition=35 mm^2 for both strains and MIC=66 µg/ml and 30 µg/ml respectively at the dose of 300 µg/ml) in comparison to other strains.. Compounds SV-13, and SV-14 showed maximum activity against *E. coli* (zone of inhibition=38 mm^2 and 45 mm^2 respectively, MIC=40 µg/ml and 30 µg/ml respectively at the dose of 300 µg/ml) in comparison to other strains used by us. Compound SV-8, SV-9, SV-11, and SV-12 showed maximum activity against *MRSA* (zone of inhibition lies between 35 mm^2 to 40 mm^2 for all these compounds, MIC=30 to 40 µg/ml at the dose of 300 µg/ml) in comparison to other strains. Compounds SV-8, SV-9, SV-10, SV-11, SV-12, SV-13 and SV-14 exhibited promising antibacterial activity against *MRSA* at the selected doses *i.e.* 300 and 100 µg/ml.

Antifungal Activity

Ofloxacin is an antibacterial drug and inactive against fungi, in order to evaluate the result of addition of different functional groups to its basic structure, the antifungal activity of its derivatives was carried out against; *A. fumigatus*, *P. carinii* and *A. niger* and results are summarized in Table 3. It was found from the result that compound SV-14 has got enhanced activity against all the antifungal strains used. The compound SV-8 also showed moderate activity against *A. niger and P. carinii.* The compound SV-10 showed moderate activity against *A. niger.*

CONCLUSION

Antimicribial activity was performed on all synthesized compounds. From all the synthesized compounds, compound compound SV-14 showed maximum activity against *H. pylori*. Compounds SV-8, and SV-14 showed maximum activity against *P. aeruginosa*. Compounds SV-13, and SV-14 showed maximum activity against *E. coli*. Compound SV-8, SV-9, SV-11, and SV-12 showed maximum activity against *MRSA*. Compounds SV-8, SV-9, SV-10, SV-11, SV-12, SV-13 and SV-14 exhibited promising antibacterial activity against *MRSA* at the selected doses.

Compound SV-14 has got enhanced activity against all the antifungal strains used. The compound SV-8 also showed moderate activity against *A. niger and P. carinii.* The compound SV-10 showed moderate activity against *A. niger.*

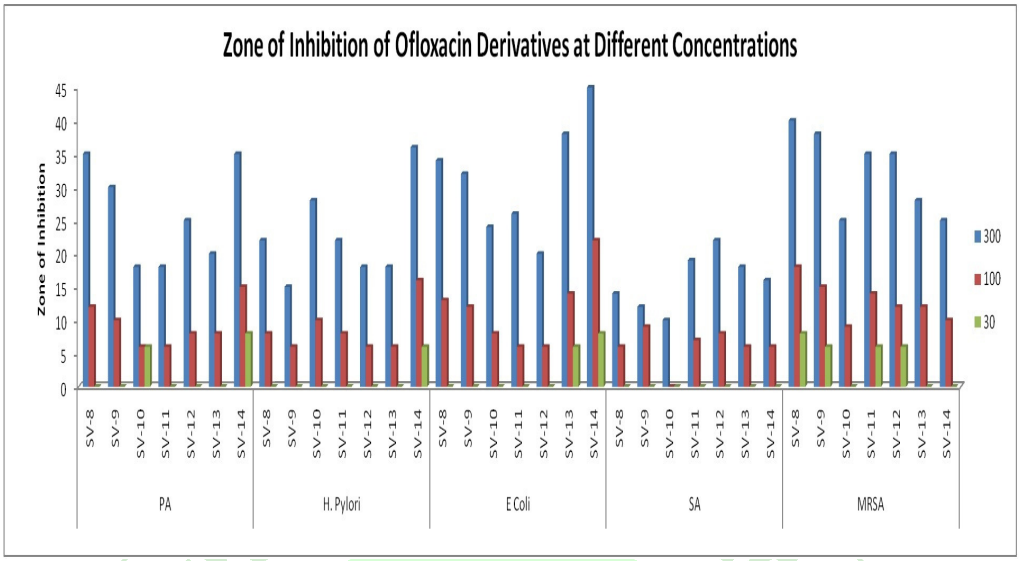


Fig. 1: Zone of Inhibition (ZOI) of the Schiff bases (SV-8 to SV-14) at Different Concentrations

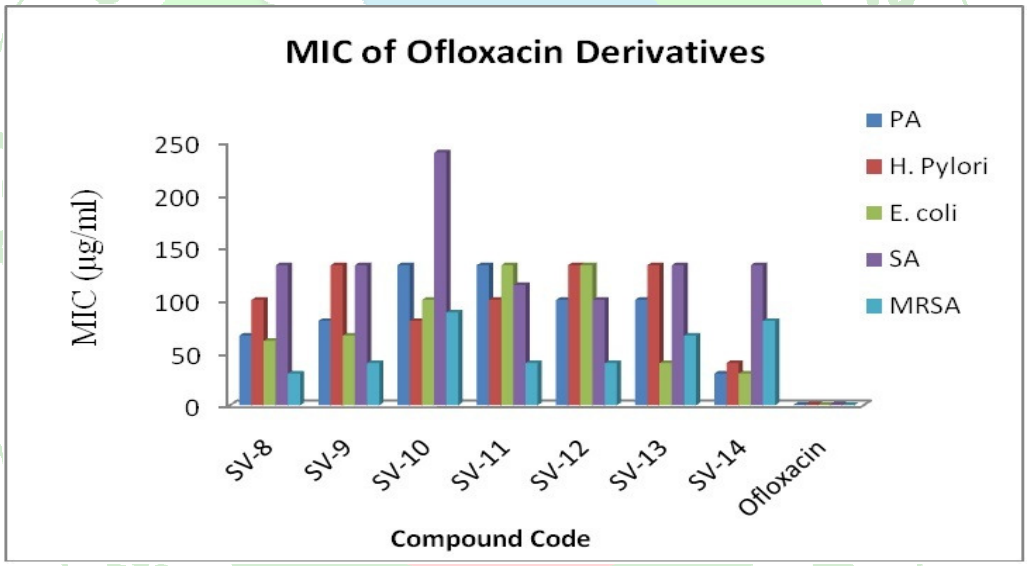


Fig. 2: Minimum Inhibitory Concentration (MIC) the Schiff bases (SV-8 to SV-14)

CONCLUSION

Antimicribial activity was performed on all synthesized compounds. From all the synthesized compounds, compound compound SV-14 showed maximum activity against *H. pylori*. Compounds SV-8, and SV-14 showed maximum activity against *P. aeruginosa*. Compounds SV-13, and SV-14 showed maximum activity against *E. coli*. Compound SV-8, SV-9, SV-11, and SV-12 showed maximum activity against *MRSA*. Compounds SV-8, SV-9, SV-10, SV-11, SV-12, SV-13 and SV-14 exhibited promising antibacterial activity against *MRSA* at the selected doses.Compound SV-14 has got enhanced activity against all the antifungal strains used. The compound SV-8 also showed moderate activity against *A. niger and P. carinii.* The compound SV-10 showed moderate activity against *A. niger*.

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