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QSAR Studies and Synthesis of C-5 Substituted Derivatives of Counter Fluoroquinolone Drugs

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Abstract : *Fluoroquinolones have attracted much attention because of their broad spectrum of activity against various bacteria, mycobacteria and parasites but there are numerous factors including solubility, side effects and cost of using these drugs. Drug discovery and development is a broad field that encompasses many disciplines like formation of a new moiety, methodology, potency, technology and green chemistry since four decades. Modification of Fluoroquinolone series (ciprofloxacin, norfloxacin, levofloxacin, sparfloxacin and pefloxacin) is the focus of present research. Firstly, fluoroquinolone derivatives had been designed with the help of computer-assisted drug designing (CADD). Secondly, 5-substituted morpholine derivatives are synthesized. Experimental analysis, FTIR and ¹H NMR spectral data evaluated that the results are in accordance with the estimated SAR and QSAR studies i.e. newly synthesized drugs are found to be more potent with increased solubility and less side effects as compared to the parent counter fluoroquinolone drugs.*

Keywords *- Fluoroquinolone drugs; Solubility; Fourier Transform Infrared Spectra, QSAR.*

I. INTRODUCTION

The fluoroquinolones are the most important antimicrobial agents that have demonstrated activity against a wide range of Gram-positive and Gram-negative bacteria and have proved useful against micro-organisms that are resistant to other antibacterial agents.[1] Some examples include ciprofloxacin, pefloxacin, levofloxacin and norfloxacin with newer ones entering the scene almost every five years. The term "QSAR" refers to quantitative structure activity relationships, which can be regarded as computer simulations of possible toxic effects of chemicals.[2] There are various applications of QSAR to drug design, some examples of which relied primarily on statistical correlation and some, on computer-based visualization and modeling. An early example of QSAR in drug design involves a series of 1-(X-phenyl)-3,3-dialkyl triazenes.[3][4] SAR/QSAR (Quantitative Structure Activity Relationship) methods are basically related to green chemistry as they allow us to screen out compounds that possess to many undesirable characteristics before investing time in preparing, analyzing and testing the compounds.[5] Ciprofloxacin, norfloxacin, levofloxacin, sparfloxacin and pefloxacin are the focus of this review.[6] Ciprofloxacin, norfloxacin and pefloxacin are the second generation fluoroquinolone while levofloxacin and sparfloxacin are the third generation fluoroquinolones with a 6-fluoro substituent and 7-piperazinyl substituent on the quinolone ring structure.[7]

The fluoroquinolones have been analyzed by various methods which have been described in different literatures.[8][9] This review has become needful in view of the rapid progress in quinolone research and development with the help of SAR/QSAR methods. The computational tools can allow a drug molecule to be constructed within the bimolecular using knowledge of the nature of its active site.[10]

II. METHODOLOGY

STEP 1: Drug Designing using SAR (Structure activity relationship) and QSAR(Quantitative structure activity relationship) methods:

According to SAR studies of ciprofloxacin, norfloxacin, levofloxacin, sparfloxacin and pefloxacin the C-5 position has an important role in activity and the hydrogen atom at this positon can be easily replaced by amine substituent to increase the biological activity of counter fluoroquinolone drugs. 5-morpholine fluoroquinolone derivatives and other analogs of 5 fluoroquinolones had been designed using computer-assisted drug designing (CADD) and QSAR studies gave Log P values. Hence, it reveals that newly designed drugs were following Lipinski rule and are more potent than parent drug by SAR modification of their chemical structures using morpholine via chlorination at C-5 position.

STEP 2: Synthesis

Step I : Synthesis of 5-substituted chloro intermediates from counter fluoroquinolone drugs. Fluoroquinolones raw drug material 3 gm with 30 ml of dioxane was taken in a round bottom two necked 50 ml flasks. Chlorine gas was bubbled in reaction mixture for 30 minutes, then a reaction mixtures was heated under refluxed conditions for one hour. Reaction mixture poured in ice cold water, filtered, dried and recrystallised with

suitable solvent systems which yielded 5-substituted chloro intermediate in good quantity with melting point less then 300ºC. Purity has been checked by Thin Layer Chromatography, Percentage nitrogen analysis, antibiotic sensitivity tests and FTIR and ^{1}H NMR spectral studies.

Step II: Synthesis of 5-Morpholine derivatives from 5-substituted chloro intermediates. 2gm 5-substituted chloro intermediates (Intermediate A, B, C, D and E) obtained from step 1 condensed with 6 ml morpholine in dioxane solvent under refluxed conditions (80°C for 2 hours on steam bath) to synthesise 5-substituted morpholine derivatives at C-5 position. The reaction mixtures poured in ice cold water, filtered, dried and recrystallized with suitable solvent. and the products yielded in good quantity. The melting points of newly synthesized compounds 5-Morpholine derivatives A, B, C, D and E are less then 250ºC. Products were analysed with the help of Thin Layer Chromatography, percentage nitrogen analysis, antibiotic sensitivity tests and FTIR and ^{1}H NMR spectral studies.

III. OBSERVATIONS

Analytical techniques used for the conformation of reactions are Thin Layer Chromatography (TLC), Percentage Nitrogen analysis and Melting point detection of counter fluoroquinolone derivatives.

Table 1. Comparison of Molecular properties for 5-substituted chloro intermediates of fluoroquinolone drugs.

Molecular Properties	A	B	C	D	D1	E
mi Log P	1.721	1.897	1.925	2.426	2.142	2.327
TPSA	74.569	69.635	75.428	98.092	99.362	65.78
Natoms	25	24	24	28	28	25
MW	365.729	352.793	393.368	424.246	408.861	367.808
nON	6	5	7	7	7	6
nOHNH	2	2	1	4	4	1
Nviolations	0	0	0	0	0	0
Nrotb	3	3	1	3	3	3
Volume	298.00	296.813	303.824	337.101	320.631	309.711

Molinspiration drug-likeness score v2010.01

	A	B	C	D	D1	E
GPCR ligand	0.26	0.22	0.24	0.26	0.25	0.14
Ion channel modulator	0.56	0.06	0.53	0.51	0.50	0.34
Kinase inhibitor	-0.04	-0.41	-0.05	0.00	0.00	0.14
Nuclear receptor ligand	-0.56	-0.13	-0.53	-0.53	-0.52	-0.52

Table 2. Comparison of Molecular properties for 5-substituted morpholine derivatives of fluoroquinolone drugs.

Molecular Properties	Derivative A	Derivative B	Derivative C	Derivative D	Derivative D1	Derivative E
mi Log P	0.991	1.167	1.28	1.93	1.413	1.597
TPSA	87.041	82.107	87.817	110.835	106.242	78.252
Natoms	30	29	29	33	33	30
MW	416.453	403.454	445.498	477.523	459.522	418.469
nON	8	7	8	9	9	8
nOHNH	2	2	1	4	4	1
Nviolations	0	0	0	0	0	0
Nrotb	4	4	2	4	4	4
Volume	363.594	361.411	371.221	401.699	398.180	374.31

Molinspiration drug-likeness score v2010.01

	Derivative A	Derivative B	Derivative C	Derivative D	Derivative D1	Derivative E
GPCR ligand	0.19	0.22	0.20	0.20	0.17	0.09
Ion channel modulator	0.33	0.06	0.33	0.26	0.23	0.15
Kinase inhibitor	-0.10	-0.41	-0.11	-0.06	-0.06	0.06
Nuclear receptor ligand	-0.51	-0.13	-0.48	-0.50	-0.50	-0.48

Table 3. Physical data of counter fluoroquinolone drugs.

Sr. no.	Name of the drugs	Molecular Formula	Recrystall-isation Solvent	Yield (%)	M.P (ºC)	Nitrogen %age (%N)	Rf value
1.	Ciprofloxacin	$C_{17}H_{18}N_3O_3F$	Methanol & toluene	55	270	12.67	0.2000
2.	Norfloxacin	$C_{16}H_{18}N_3O_3F$	Methanol & toluene	58	221	13.15	0.2413
3.	Levofloxacin	$C_{18}H_{20}N_3O_4F$	Methanol & toluene	56	218	11.62	0.2230
4.	Sparfloxacin	$C_{19}H_{22}N_4O_3F_2$	Methanol & toluene	54	267	14.27	0.2339
5.	Pefloxacin	$C_{17}H_{20}N_3O_3F$	Methanol & toluene	59	271	12.59	0.2090

Table 4. Physical data of synthesized 5-substituted chloro intermediates of counter fluoroquinolone drugs.

Sr. no.	5-substituted chloro intermediate of	Molecular Formula	Recrystall-ising Solvent	Yield (%)	M.P (ºC)	Nitrogen %age (%N)	Rf value
1.	Ciprofloxacin	$C_{17}H_{17}N_3O_3FCl$	Methanol & toluene	52	230	11.55	0.2307
2.	Norfloxacin	$C_{16}H_{17}N_3O_3FCl$	Methanol & toluene	55	195	11.95	0.2818
3.	Levofloxacin	$C_{18}H_{19}N_3O_4FCl$	Methanol & toluene	54	190	10.67	0.2580
4.	Sparfloxacin	$C_{19}H_{20}N_4O_3F_2Cl$	Methanol & toluene	51	215	9.89	0.2705
5.	Pefloxacin	$C_{17}H_{19}N_3O_3FCl$	Methanol & toluene	57	231	11.49	0.2327

Table 5. Physical data of synthesized 5-substituted morpholine derivatives of counter fluoroquinolone drugs.

Sr. no.	5-morpholine derivative of	Molecular Formula	Recrystall-isation Solvent	Yield (%)	M.P (ºC)	Nitrogen % age (%N)	Rf value
1.	Ciprofloxacin	$C_{21}H_{26}N_4O_4F$	Methanol & toluene	50	200	13.44	0.1692
2.	Norfloxacin	$C_{20}H_{26}N_4O_4F$	Methanol & toluene	52	170	13.84	0.1909
3.	Levofloxacin	$C_{22}H_{28}N_4O_5F$	Methanol & toluene	51	165	12.54	0.1635
4.	Sparfloxacin	$C_{23}H_{29}N_4O_3F_2$	Methanol & toluene	48	170	11.72	0.1745

5.	Pefloxacin	$C_{21}H_{19}N_4O_4F$	Methanol & toluene	53	205	13.38	0.1532

Table 6. Comparison of biological activity of fluoroquinolone drugs and their C-5 derivatives.

Sr.no.	Name of the drug	Raw drug material	5-substituted chloro intermediate	5-substituted morpholine derivative
1.	Ciprofloxacin	2.3	1.72	0.99
2.	Norfloxacin	2.1	1.89	1.16
3.	Levofloxacin	2.1	1.92	1.28
4.	Sparfloxacin	2.5	2.42	1.93
5.	Pefloxacin	2.4	2.32	1.59

Table 7. Comparison of activity against soil bacteria for fluoroquinolone derivatives.

Sr.no.	Fluoroquinolone Derivatives	Strength (µg / disc)	Zone of inhibition (in mm)
1.	Derivative A	5	33
2.	Derivative B	5	32
3.	Derivative C	5	34
4.	Derivative D	5	33
5.	Derivative E	5	34

Table 8. Observed FTIR peaks of 5-substituted morpholine derivative of Ciprofloxacin (Derivative A)

Peaks(cm1) Groups	Peak assignment
3500- 3450	Hydroxyl group O- H stretching vibration, intermolecular H- bonded
3000- 2950	Aromatic,cyclic enes υ=CH & Ar- H
2900	Cyclopropyl group C- H stretching vibration
1750- 1700	CO group of acid C=O stretching vibration
1650- 1600	Quinolines δN- H bending vibration
1450- 1400	Carbonyl group υC- O
1300- 1250	Hydroxyl group δO- H bending vibration
1050- 1000	Fluorine group C- F stretching

Table 9. Observed FTIR Peaks of 5-substituted morpholine derivative of Norfloxacin (Derivative B)

Peaks(cm1) Groups	Peak assignment
3550- 3500	Hydroxyl group, intermolecular H - bonding by single bridge
3500- 3300	Imino- moity of Piperazinyl groups, NH stretching vibration
3000- 2950	Aromatic,cyclic enes υ=CH & Ar- H
2750- 2700	Ethyl group υCH3 of C2H5
2500	Acid group υOH group
1700	Carbonyl of acids υC=O stretching vibration
950- 900	Amines δNH bending vibration
1650- 1600	Quinolones υN- H bending vibration
1500- 1450	O- C- O group of acid υs stretching vibration of O- C- O group
800	Aromatic m – distribution δAr- H
1300- 1250	Hydroxyl group δO- H bending vibration
1050- 1000	C- F groups υC- F

Table 10. Observed FTIR Peaks of 5-substituted morpholine derivative of Levofloxacin (Derivative C)

Peaks(cm1)

Groups Peak assignment

3500- 3450	Hydroxyl group O- H stretching vibration, intermolecular H- bonded
3000- 2950	Aromatic,cyclic enes υ=CH & Ar- H
1650- 1600	Quinolines δN- H bending vibration
1450- 1400	Carbonyl group υC- O
1300- 1250	Hydroxyl group δO- H bending vibration
1050- 1000	Fluorine group C- F stretching
2750- 2700	Methyl group υCH3
2500	Acid group υOH group
1500- 1450	O- C- O group of acid υs stretching vibration of O- C- O group
950- 900	Amines δNH bending vibration

Table 11. Observed FTIR Peaks of 5-substituted morpholine derivative of Sparfloxacin (Derivative D)

Peaks(cm1)

Groups Peak assignment

3500- 3450	Hydroxyl group O- H stretching vibration, intremolecular H- bonded
3400- 3350	Imino- moity of Piperazinyl groups, NH stretching vibration
3000- 2950	Aromatic,cyclic enes υ=CH & Ar- H
2900	Cyclopropyl group C- H stretching vibration
1750- 1700	C=O group of acids υC=O stretching vibration
1650- 1600	Quinolines δN- H bending vibration
1550- 1500	Alkyl groups υCH3 and υCH2
1400- 1350	Hydroxyl group δO- H bending vibration
1250- 1200	Oxo group C- O- C stretching vibration
1050- 1000	Fluorine group C- F stretching

Table 12. Observed FTIR Peaks of 5-substituted morpholine derivative of Pefloxacin (Derivative E)

Peaks(cm1)

Groups Peak assignment

3050- 3000	Hydroxyl group O- H stretching vibration, intremolecular H- bonded
3000- 2950	Aromatic,cyclic enes υ=CH & Ar- H
2750	Alkyl groups υCH3
1750- 1700	C=O group of acids υC=O stretching vibration
1650- 1600	Quinolines δN- H bending vibration
1550- 1500	Alkyl groups υCH3 and υCH2
1450- 1400	Methylene group in Benzoxazine stretching vibration of CH2
1400- 1350	Hydroxyl group δO- H bending vibration
1250- 1200	Oxo group C- O- C stretching vibration
1050- 1000	Fluorine group C- F stretching
950- 800	Aromatics & enes =C- H out of plane bending vibration

Table 13. ^{1}H NMR spectral data:

C-5 proton of fluoroquinolone drugs observed at 5.9 ppm. In case of 5-substituted chloro intermediates and 5-substituted morpholine derivatives C-5 proton was absent.

	Raw drug material	5-substituted chloro intermediates	5-substituted morpholine derivatives
C-5 Proton (^{1}H)	Present	Absent	Absent

IV. RESULT

In the present work, SAR and QSAR methods were used for the designing of 5-substituted fluoroquinolone derivatives. SAR and QSAR studies of counter fluoroquinolone drugs revealed that C-5 has

hydrogen atom which could be replaced by amine moiety. Some type of bulky (5- or 6- membered rings) nitrogen heterocycle e.g. morpholine offers the best enhancement of activity. Literature reveals that morpholine relieves pain and produce euphorbia. There are receptors to which morpholine binds in brain, spinal cord and gastro intestinal tract. So, C-5 position has been replaced by morpholine to increase the solubility and biological activity according to Lipinski's rule. Comparison of Molecular properties for 5-substituted chloro intermediates of fluoroquinolone drugs has been demonstrated in Table 1 and the comparison of molecular properties for 5-substituted morpholine derivatives of fluoroquinolone drugs is demonstrated in Table 2.

Physical data of synthesized 5-substituted chloro intermediates of counter fluoroquinolone drugs and synthesized 5-substituted morpholine derivatives of counter fluoroquinolone drugs is reported in Table 4 and Table 5. These observations prompted to optimize the reaction conditions and the method was finally optimized to get very high yield of the product having good qualities which required minimum efforts, solvents and chemicals to purify the products. Biological activity of fluoroquinolone drugs and their C-5 derivatives derivatives has been compared (Table 6) in which C-5 derivatives are showing increased biological activity according to Lipinski's rule as the Log P values of C-5 derivatives are less as compared to the parent fluoroquinolone drugs and hence C-5 substituted morpholine derivatives are more potent.

All the synthesized C-5 derivatives were initially tested for their antibacterial activity using dose of 5 µgm/disc. This was done identify the activity of fluoroquinolone derivatives against bacteria. The results (Table 8) show a few compounds to be comparably more active against the bacterial culture. Then the selected more potent compounds were further analysed using FTIR and ^{1}H NMR.

Observed FTIR peaks of 5-substituted morpholine derivatives (Derivative A, Derivative B, Derivative C, Derivative D and Derivative E) are reported in Table 9, 10, 11 and 12. According to ^{1}H NMR spectral data C-5 proton of fluoroquinolone drugs observed at 5.9 ppm. was absent in case of 5-substituted chloro intermediates and 5-substituted morpholine derivatives.

V. CONCLUSION

The quinolone class of antimicrobial agents has been developed and grown within this time frame. Despite ongoing development of new agents in this important class, resistance to newly released agents continues to be observed. Recent data suggests emerging resistance is specifically linked to use of some of the older compounds. We have learned much about how structural modifications affect both activity and toxicity. A recent report assessed the toxicity profiles of newer fluoroquinolone agents.

Despite these favourable properties, the earlier fluoroquinolones had limited potency against some clinically important organisms, especially Gram-positive pathogens so that the development of resistance to these organisms has become a serious problem. Thus the development of new fluoroquinolones with a better pharmacokinetic profile, potency, broad spectrum of activity, solubility, prolonged serum half-life and oral and parenteral routes of administration has been a major focus on recent research. The increasing interest in this class led me to review the promising new fluoroquinolones in clinical trials.

Structure-activity relationships (SAR) and quantitative SAR (QSAR) studies have been extensively used to correlate molecular structures to their biological activities. A primary goal of QSAR/SAR methods is to find rules, which can lead to reliable classifications, and predictions of the biological activity for tested, untested or hypothetical compounds. The obtained information can be used for the selection or design of better structure. It has been estimated that over 10 000 analogues of nalidixic acid or the fluoroquinolones have now been synthesised. The benefits of some of these new compounds include: oral and parenteral dosing, a much broader spectrum of antibacterial activity, good tissue distribution, improved pharmacokinetic profiles, stability and a comparatively low incidence of adverse effects. This review considers the structure of the core fluoroquinolone molecule, some of the changes that feature on current class members under development, and the effects that these chemical modifications may have on the interaction of these compounds with man.

The results obtained from QSAR study consider not only wide range of structures, but also various physic-chemical interactions involved in enzyme inhibitor complex. Experimental analysis, Fourier transform infrared (FTIR) and ^{1}H NMR spectral data, antibiotic sensitivity tests and QSAR studies evaluated that the biological activity of the newly synthesized drugs i.e. 5-substituted morpholine derivatives of ciprofloxacin, levofloxacin and sparfloxacin was found more potent and hence concluded that these novel compounds were following Lipinski's rules and were more active than the parent counter fluoroquinolone drugs.

The results obtained are in agreement with the observed QSAR results of fluoroquinolones. So, besides of the antibacterial activity of newly synthesized drugs, solubility was more remarkable with less side effects. As this research was focused on the synthesis of 5 fluoroquinolone derivatives, it provides an insight into a variety of approaches resulting in elegant manipulations of their basic skeleton and some breakthroughs in the synthetic strategies of a widely used drugs and these have immensely helped in accelerating their market growth as well as continuing research for newer fluoroquinolones.

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