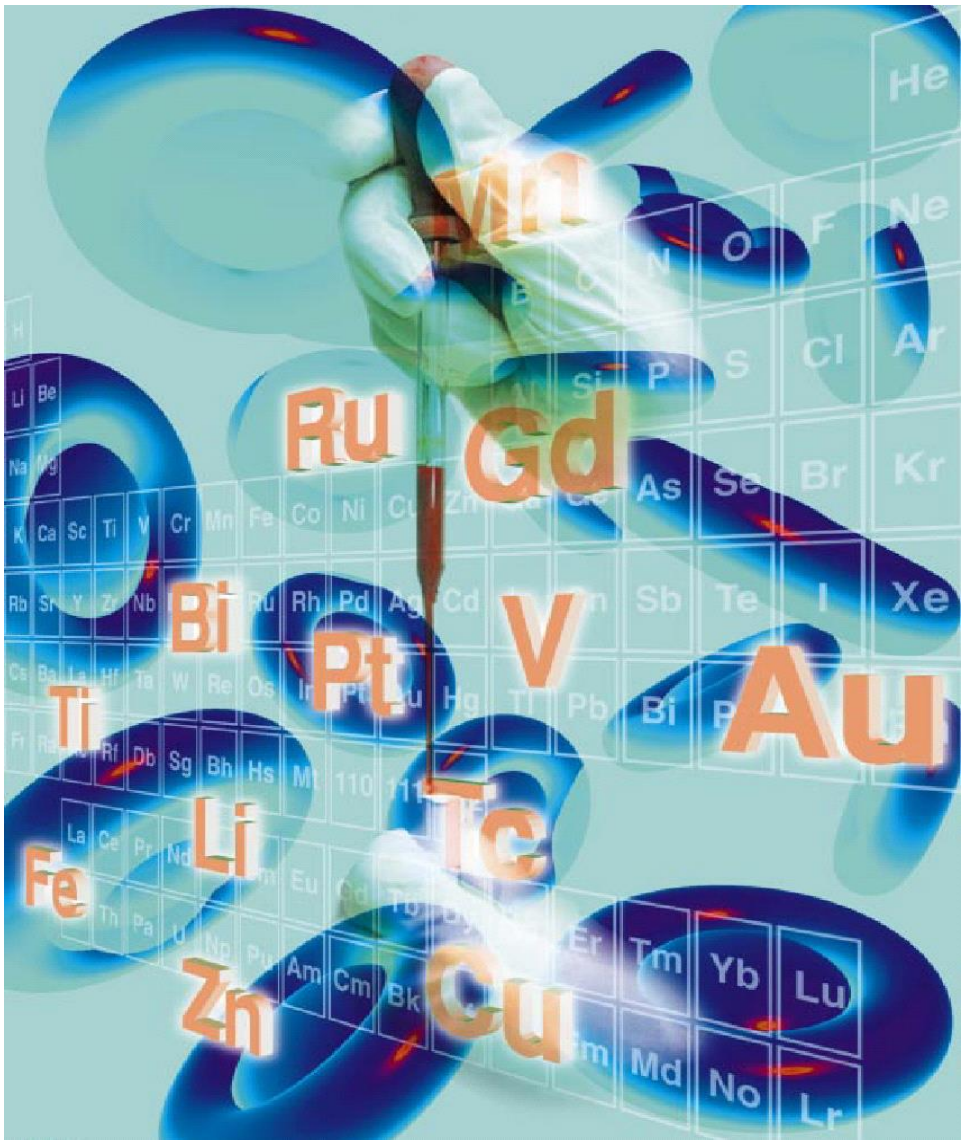
Chapter 1

Introduction



1.1 Introduction

Over a few decades ago, a new milestone in the field of antibacterial drug discovery was reached by the introduction of a novel class of molecules, known as quinolones. In the past few years quinolones comprised a relatively large growing and most interesting group of antibacterials in the field of antibacterial chemotherapy This is because they potentially offer many of the attributes of an antibiotic, combining high potency, broad intravenous formulations, high serum levels, and potentially low incidence of side effects [1-3].

The first quinolone, nalidixic acid (1-ethyl-7-methyl-1,4-dihydro-4-oxo-1,8 naphthyridine-3-carboxylic acid) was introduced in 1962. The substance was discovered by George Lesher and coworkers in a distillate during chloroquine synthesis (Fig 1.1). It demonstrated anti-Gram negative antibacterial activity, and possessed pharmacokinetic properties for treating urinary tract infections [4-7].

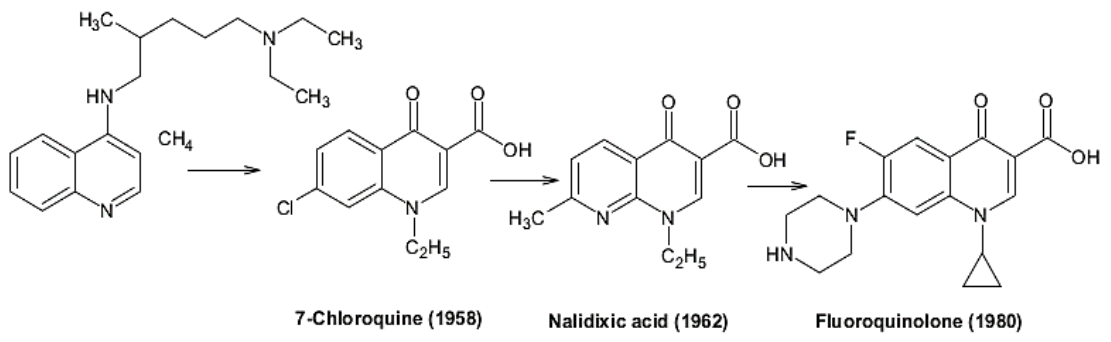


Fig 1.1: Sequential landmarks in the development of quinolones.

The clinical use of nalidixic acid was limited by its narrow spectrum of activity. Several modifications were made on the basic nucleus in order to enlarge the antibacterial spectrum and to improve the pharmacokinetics properties, two of these considered as being major are : introduction of a piperazine moiety or another N-heterocycle in the position 7 and introduction of a fluorine atom at the position 6

(fluoroquinolones). Taking into account the basic nucleus and spectrum of their antimicrobial activity quinolones are classified into four generations (Fig 1.2).

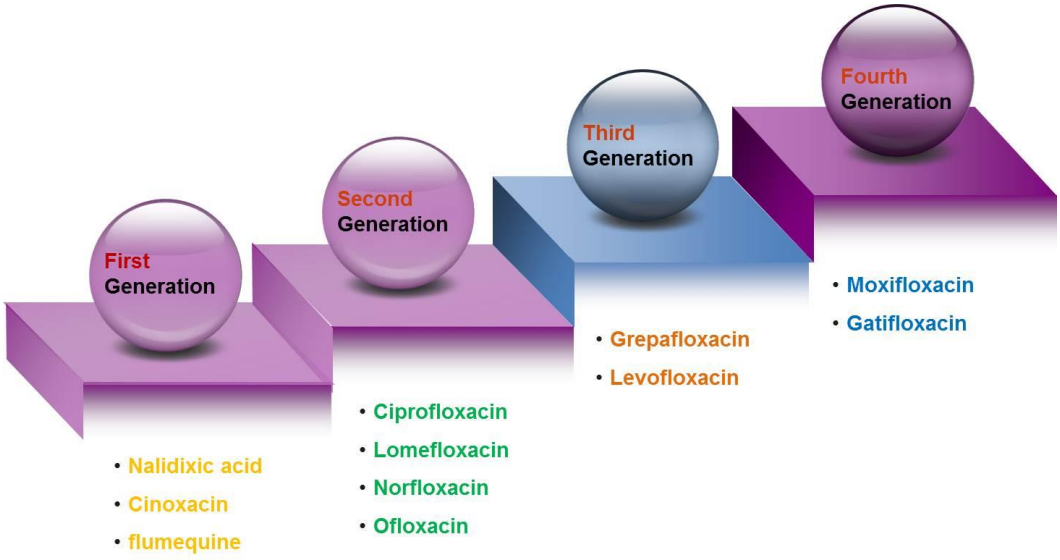
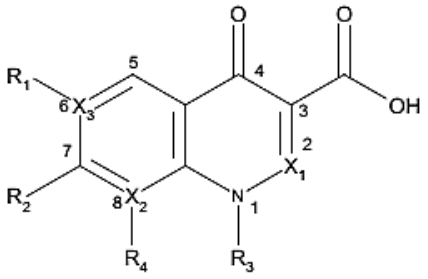


Fig 1.2: Classification of Quinolones

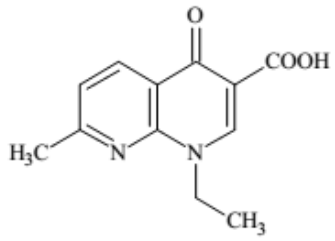
First generation quinolones

The first generations of quinolone derivatives reflect the early experimentation with the original nalidixic acid structure. For example, all of these quinolones retain a nitrogen atom at the C-1 position while modifying the naphthyridone structure of nalidixic acid. Nalidixic acid **II,** cinoxacin **III**, flumequine **IV** and 7-piperazinyl derivative, pipemidic acid **V** are the first generation quinolones. These compounds attain high concentrations in the urinary tract and hence are therapeutically useful for the treatment of urinary tract infections. Cinnoxacin and nalidixic acid require more frequent dosing than the newer quinolones, as they achieve minimum serum level and are more susceptible to the development of bacterial resistance.



I

Fig 1.3: General structure of Quinolones



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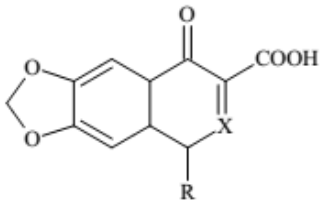

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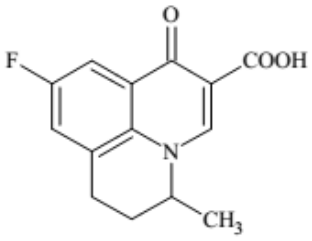

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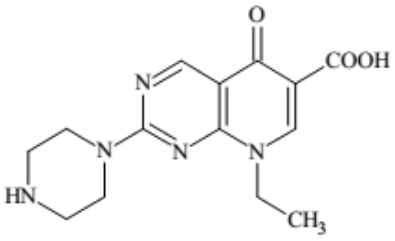
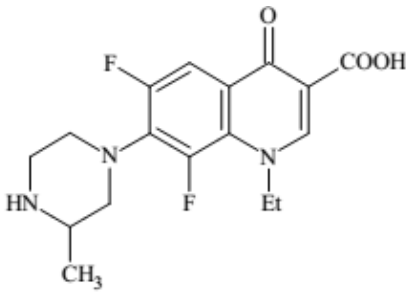


V

Second generation quinolones

Second generation quinolones have expanded Gram negative activity but limited Gram positive activity. These agents are most active against aerobic Gram negative bacilli. The fluoroquinolones lomefloxacin **VI,** norfloxacin **VII** and enoxacin **VIII** are the class-I agents of 2nd generation.



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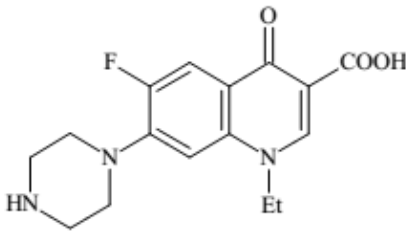

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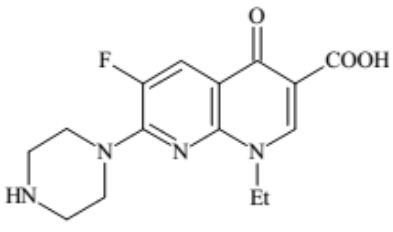

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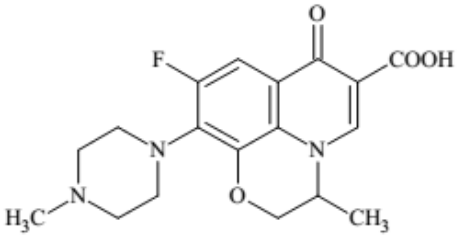
Ofloxacin I**X** and ciprofloxacin **X** are the class-II agents of the 2nd generation achieving higher serum, tissue and intracellular drug concentration compared with class I agents.



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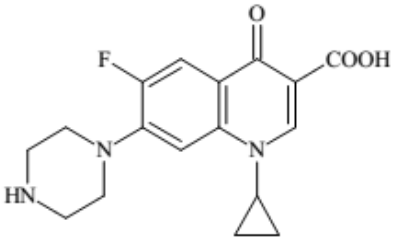
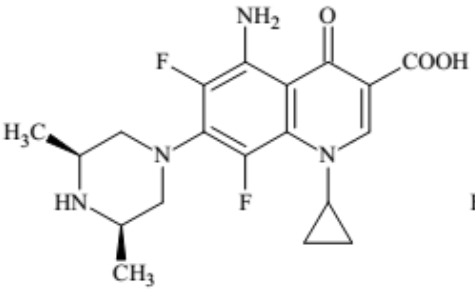


X

Third generation quinolones

The fluoroquinolones sparfloxacin **XI**, gatifloxacin **XII**, levofloxacin **XIII** and moxifloxacin **XIV** are the third generation quinolones having expanded Gram negative and improved Gram positive activity.



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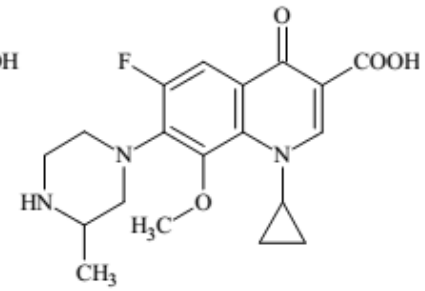

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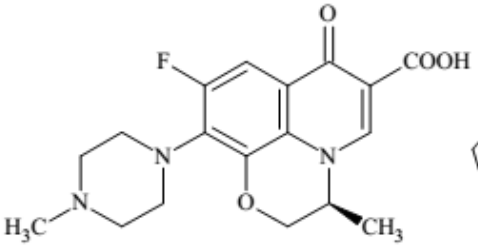

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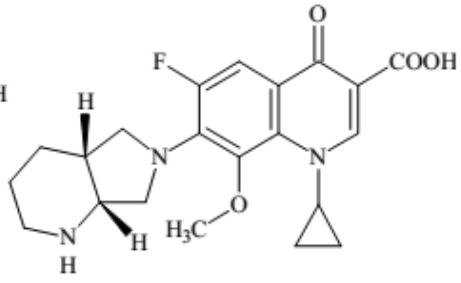
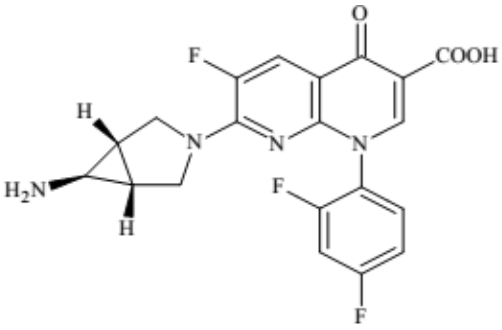


XIV

Fourth generation quinolones

Fourth generation quinolones include trovafloxacin **XV** and Clinafloxacin **XVI**. These new fluoroquinolones have significant antimicrobial activity against anaerobes and pneumococcus while maintaining the gram-positive and gram-negative activity as well as activity against Pseudomonas species [8-13].



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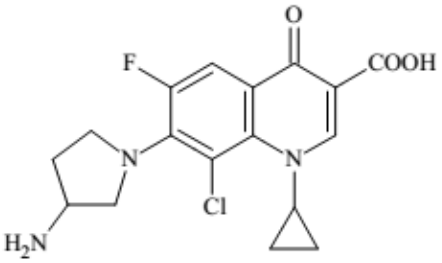

XVI

1.2 Functional domains of Quinolone pharmacophore

Molecular structure of quinolone is divided into certain domains depending upon their functions . The basic unit consists of pyridone ring and a carboxyl group. In this unit the pyridone nitrogen (N-1) is the integral part, the groups attached can be varied; the carboxyl at C-3 position is replaceable by a fused thiazolidone, while very few C-4 variations have been reported [14].

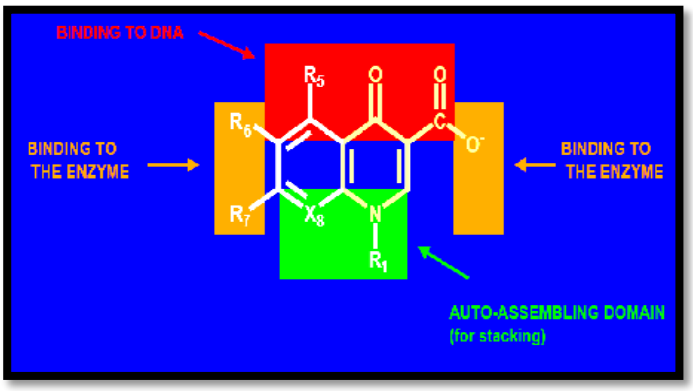

Fig 1.4: Structure activity relationship of quinoloes.

The organic chemists have successively been trying to enhance the biological activity of quinolones against Gram-positive as well as Gram-negative bacteria by varying the substitutions at different

positions like N-1, C-2, C-3, C-5, C-6, C-8 (Fig 1.3) and primarily at C-7 with small groups like H, CH_3, F, cyclopropyl etc. to bulky moieties like benzoxazines.

Quinolone moiety can be divided in four major regions((Fig. 1.4). The upper margin or the "northern front" of the quinolones consisting of pharmacophoric carboxyl group at C-3 and the ketonic group at C-4 is complementary to the DNA bases exposed and is suitable for hydrogen bonding with DNA. It is likely that certain substituents at C-5 may also contribute to this. The lower margin (southern front) of the molecule is considered to be a lipophilic and self-association region consisting of N-1 with its pendant substituents. The basic substituents at C-7 serve to orient the quinolone molecules so that carboxyl and protonated amino groups line up near each other in the adjoining quinolone molecules in the vertical and horizontal directions and hence are thought to contribute to their binding on the B subunit of the DNA gyrase.

Thus, the proposed model suggests three functional domains on the quinolone molecules: the DNA-binding domain, the drug self-association domain and the drug-enzyme interaction domain. Such functional domains for the quinolone derivatives provide a guideline for synthesizing improved drugs with novel structures. For example, the substitution of groups with capabilities of generating amphoteric quinolones tends to yield more potent compounds.

Although the structure-activity relationships of fluoroquinolones have been extensively investigated, the optimum substituent at the C-7 position, which has a great impact on potency, spectrum, solubility, and pharmaco-kinetics, has not been precisely defined. The most extensively investigated substituents are piperazin-1-yl and its 4-substituted derivatives. For example, pefloxacin, the 4-methyl-norfloxacin, and other 4-substituted piperazin-1-yl prodrugs of norfloxacin were prepared to improve the bioavailability of the parent [15].

1.3 Mechanism of Action

The biological targets for the quinolones are bacterial topoisomerase II, known as DNA gyrase and bacterial topoisomerase IV. Both these enzymes are type II topoisomerases since they temporarily cleave both strands of duplex DNA and reseal them following passage of an uncut portion of the molecule through the temporary gap [16]. The net result is a change in the topology of the DNA molecule. Since most of the functional features of DNA are topologically dependent, these enzymes are essential for transcription, translation, repair and storage processes. Both the enzymes are vital for bacterial survival and are very widely distributed, which rationalizes the broad antibacterial spectrum for the fluoroquinolones. Inhibition of either enzyme by quinolones can be lethal to bacteria. It is proposed that the fluoroquinolones form a ternary complex consisting of the drug, DNA and enzyme that interferes with DNA transcription, replication and repair and promotes its cleavage, leading to bacterial cell death (Fig. 1.5) [15,17]. The interaction of DNA with enzyme forms a pocket for binding of the quinolone drugs.

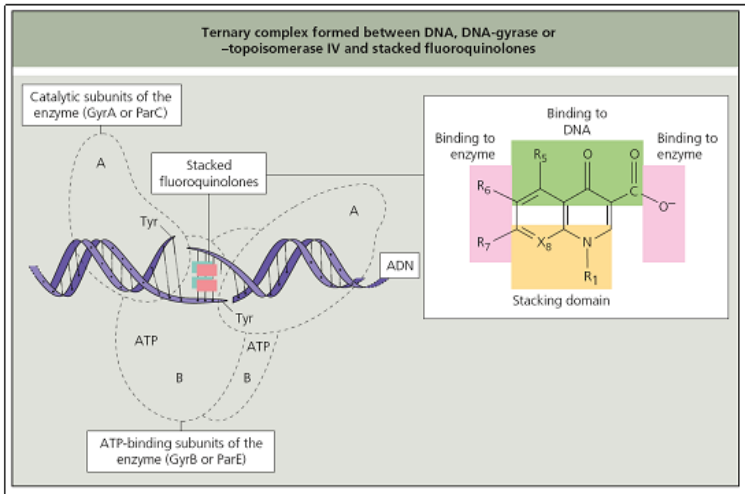

Fig 1.5: Ternary complex formed between DNA, DNA gyrase and stacked fluoroquinolones.

1.3.1 Quinolones: Antibacterial to Anticancer agents

Quinolones are a very important family of antibacterial agents, they corrupt the activities of prokaryotic type II topoisomerases, DNA gyrase and topo isomerase IV, and induce them to kill cells by generating high levels of double-stranded DNA breaks. Type II topoisomerases modulate the topological state of the

genetic material by passing an intact DNA helix through a transient double stranded break that they generate in a separate DNA segment [18]. Like bacterial cells, eukaryotic species require a type II topoisomerase, for viability[19]. Beyond its required physiological functions, the enzyme is a target for some of the most active compounds currently employed for the treatment of human cancers [20,21]. In view of the mechanistic similarities and sequence homologies exhibited by the prokaryotic type II topoisomerases and the eukaryotic type II topoisomerases, tentative efforts to selectively shift from an antibacterial to an antitumoral activity was made by synthesizing novel classes of quinolones that displayed significant activity against malignant neoplasm. In addition to the antibacterial quinolones, specific members of quinolone family display high activity against eukaryotic type II topoisomerases, as well as cultured mammalian cells and in vivo tumor models [22]. These anticancer quinolones represent a potentially important source of new anticancer agents.

1.4 Role of metal ions in quinolone chemistry

The interactions between the DNA unpaired bases and the quinolones is well explained by the quinolone drug-DNA model. Mg (II) ion acts as a bridge between the quinolone and the phosphate group of the DNA (Fig. 1.6).This DNA-quinolone-Mg (II) complex as a whole is stabilized by the stacking interactions between the condensed rings of the drug and the DNA bases [23, 24].Further studies also suggest that this DNA-quinolone-Mg (II) complex is responsible for poisoning the gyrase-DNA complex. The conformational equilibria of the DNA gyrase in the presence of Mg (II) and ciprofloxacin have been studied and it is proposed that the magnesium mediated quinolone binding to the enzyme might be involved in the mechanism of action of the quinolone drugs [25]. Robles and coworkers have carried out a detailed theoretical study on the structure and activity of certain quinolones and the interaction of their Cu (II) complexes with DNA. They suggested that the

intercalation of the metal-quinolone complex is an important step in the quinolone action [26].All these evidences indicate that the role of metal ions is imperative for the function of fluoroquinolones.

The synthesis and characterization of new metal complexes with fluoroquinolones are of great importance for better understanding the drug–metal ion interactions. It was suggested that the reactions of metal ions with fluoroquinolones were essential for the activity of these antimicrobial agents, and the metal ions (magnesium, copper, and iron) may bridge the binding of the quinolone to DNA gyrase or bacterial DNA directly.

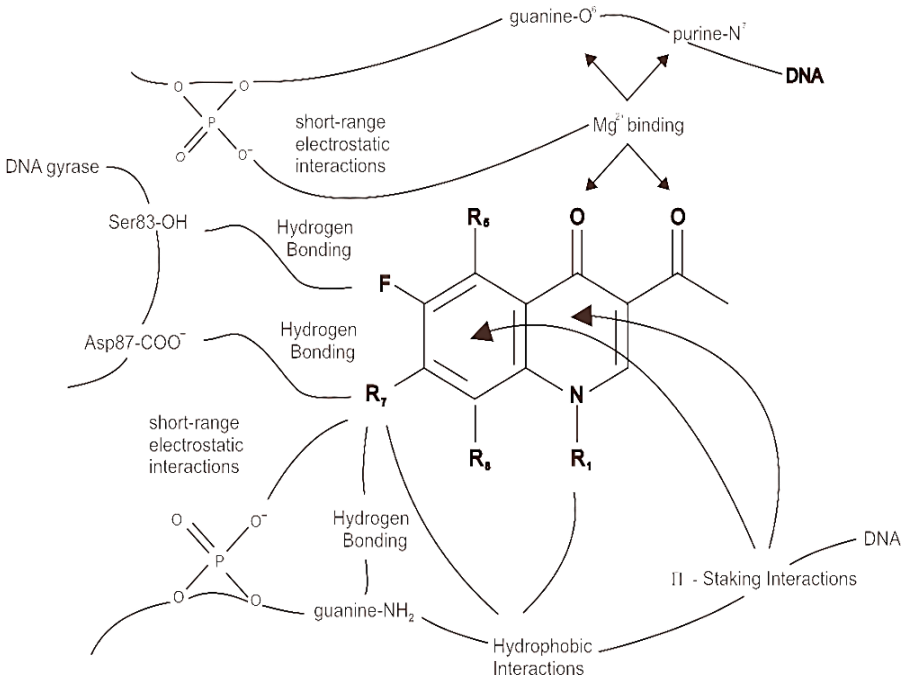

Fig 1.6 : DNA-Quinoloe-Mg^{2+} Complex

1.5 Chemical properties of quinolones related to complexation Process

Most quinolone molecules are zwitterionic, based on the presence of a carboxylic acid function at the 3rdposition and a basic piperazinyl ring (or another N-heterocycle) at the 7thposition. Both functions are weak and give a good solubility for the quinolones in acidic or basic media. Protonation equilibria of quinolones have been studied in aqueous solution using potentiometry, ^{1}H-NMR and UV spectrophotometry [27, 28]. For a quinolone molecule with the general structure depicted in Fig. 1.7, two proton-binding sites can be identified. In solution, such a molecule exists in four microscopic protonation forms, two of the microspecies being protonation isomers. The microconstants describe the proton binding affinity of the individual functional groups and are used in calculating the concentrations of different protonation isomers depending on the pH. The quinolones exist mainly in the zwitterionic form between pH 3 and 11.

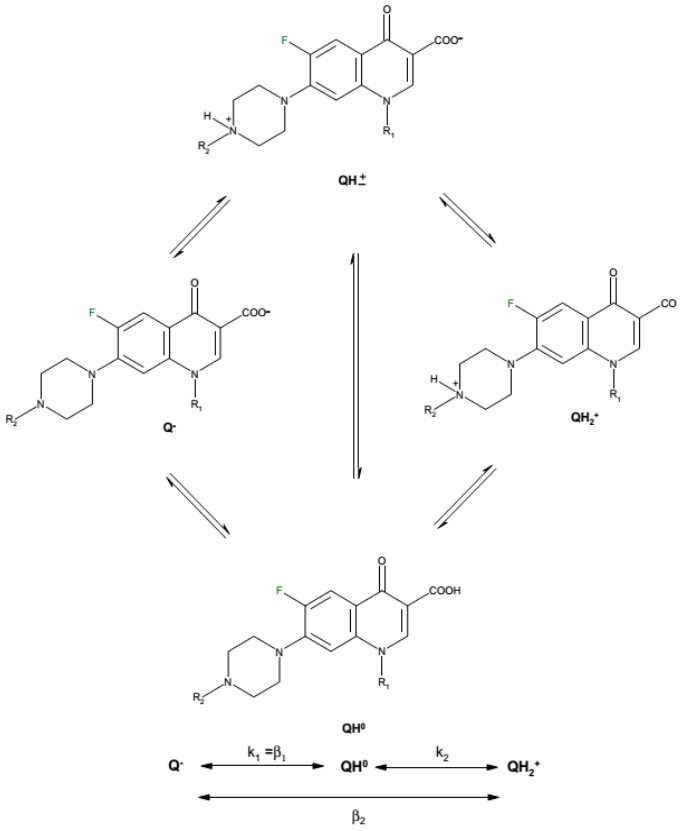

Fig 1.7: Protonation scheme of a fluoroquinolone molecule with piperazine ring at the 7-position.

Quinolone microspeciation has been correlated with bioavailability of quinolone molecules, serum protein binding and antibacterial activity [20]. The microspeciation is also important in the synthesis of metal complexes, the quinolone molecules acting as ligand in the deprotonated form (Q^-) in basic conditions, and in the zwitterionic form ($QH^{\pm}$) at pH 7.0 (neutral). In strongly acidic medium, quinolones form ionic complexes in their cation form (QH_2^+).

In the metal complexes, quinolones can act as bidentate ligand, as unidentateligand and as bridging ligand. Frequently, the quinolones are coordinated in a bidentate manner, through one of the oxygen atoms of deprotonated carboxylic group and the ring carbonyl oxygen atom [Fig. 1.8(a)]. Rarely, quinolones can act as bidentate ligand coordinated via two carboxyl oxygen atoms [Fig. 1.8(b)] or through both piperazinic nitrogen atoms [Fig. 1.8(c)]. Quinolones can also form complexes as unidentate ligand coordinated to the metal ion through terminal piperazinyl nitrogen [Fig. 1.8(d)]. In the polymeric complexes in solid state, multiple modes of coordination are simultaneously possible.

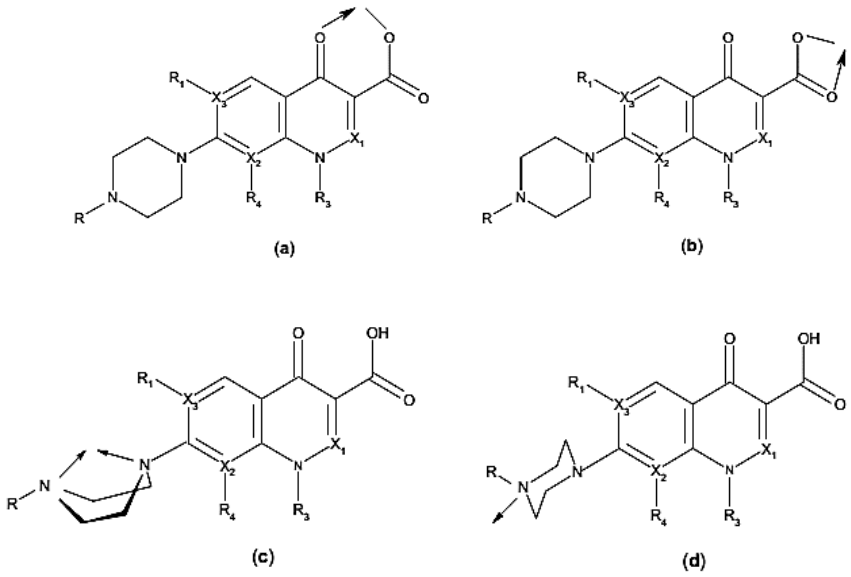

Fig 1.8: Main coordination modes of quinolones

1.6 Metals and metal complexes in medicine

The medicinal uses and applications of metals and metal complexes are of increasing clinical and commercial importance. Monographs and major reviews, as well as dedicated volumes, testify to the growing importance of the discipline [29-39]. In medicinal chemistry, traditionally dominated by organic compounds, metal complexes have gained favour as diagnostic tools and therapeutic agents.

Although metals have been used throughout human history in treating various pathological disorders, it has only been since the landmark discovery of cisplatin in the 1960's that the full impact of metal based compounds in the treatment of cancer has been fully realized. Metal-containing compounds offer many advantages over conventional carbon-based compounds in the development of new medicines. These advantages are due to their ability to coordinate ligands in a three dimensional configuration, thus allowing functionalization of groups that can be tailored to defined molecular targets [40, 41]. Metal based complexes offer a rich environment to build upon a variety of distinct molecular structures that confer a wide spectrum of coordination numbers and geometries, as well as kinetic properties, that cannot be realized with conventional carbon-based compounds [42, 43]. The partially filled d-orbitals in transition metals impart interesting electronic properties that can act as suitable probes in the design of anticancer agents [44]. The oxidation state of a metal is also an important consideration in the design of coordination compounds, given that it allows the participation in biological redox chemistry and plays an influential role in optimal dose and bioavailability of the agent administered [45,46]. Furthermore, the ability to undergo ligand exchanged reactions offers a myriad of opportunities for metals to interact and coordinate to biological molecules, as demonstrated by the widely used drug cisplatin [40].

The success of metal based pharmaceuticals, such as cis–platin and silver sulfadiazine (SSD), is significant in rising interest in the design of metal compounds as drugs and diagnostic agents. Investigations in this area focus mostly on possible interactions of

these metal ions with diverse biomolecules, in an effort to contribute to future development of new therapeutics or diagnostic agents. A wide range of metal complexes are already in clinical use, and encourage further studies for new metallodrugs, such as metal mediated antibiotics, antibacterial, antiviral, antiparasitic, radiosensitizing agents and anticancer compounds.

1.7 DNA interactions of metal complexes

DNA is the pharmacological target of many of the drugs that are currently in clinical use or in advanced clinical trials. Targeting DNA to regulate cell functions by modulating transcription (gene expression and protein synthesis) or by interfering with replication (a major step in cell growth and division) seems logical, intuitively appealing and conceptually straightforward. Small ligand molecules bind to DNA and artificially alter and/or inhibit the functioning of DNA. These small ligand molecules act as drug when alteration or inhibition of DNA function is required to cure or control a disease [47].

The study of interaction of drugs with DNA is very exciting and significant not only in understanding the mechanism of interaction, but also for the design of new drugs. However, mechanism of interactions between drug molecules and DNA is still relatively little known. It is necessary to introduce more simple methods for investigating the mechanism of interaction. By understanding the mechanism of interaction, designing of new DNA-targeted drugs and the screening of these in vitro will be possible [48].

There are two modes of drug–DNA binding, covalent and noncovalent.

1.7.1 Covalent mode of binding

Many anticancer drugs in clinical use function by interacting with DNA, not only those that bind to DNA through non-covalent interactions but also those that form covalent adducts such as via alkylation or inter- and intrastrand crosslinking [49].

The covalent mode of drug–DNA binding is irreversible and invariably causes the complete inhibition of DNA processes and subsequent cell death (if the reaction is not directly chemically reversible). A major advantage of covalent binders is their high binding strength.

Moreover, covalent bulky adducts can cause DNA backbone distortion, which in turn can affect both transcription and replication [50]. Cis-platin [cis-dichloro diammine platinum(II)] is a famous covalent binder used as an anticancer drug, and makes an intra/interstrand cross-link through the metal centre with the nitrogens on the DNA bases (Fig. 1.9). The covalent attachment of organic intercalators to transition metal complexes, producing metallointercalators, can lead to novel DNA interaction that affect biological activity. Metal complexes having σ-bonded aromatic side arms can act as dual-function complexes: they can bind to DNA both by metal coordination and through intercalation of the attached aromatic ligand. These aromatic side arms introduce new mode of DNA binding, involving mutual interactions of functional groups held in close proximity [51]. The covalent binders are also called alkylating agents due to adduct formation because they are used in cancer treatment to attach an alkyl group (C_nH_{2n+1}) to DNA [52].

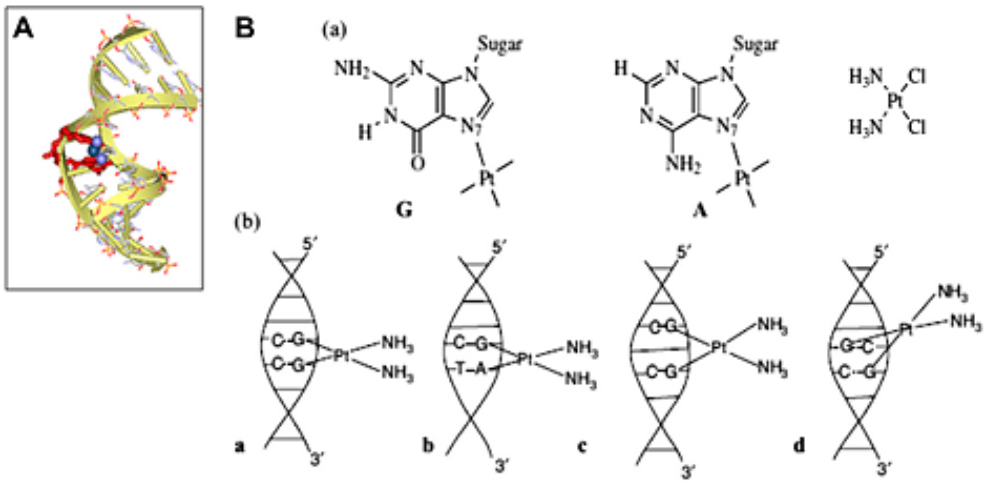

Fig. 1.9(a): Cisplatin covalently bonded to DNA. B. (a) Modes of binding of cisplatin to guanine (G) and adenine (A); (b) 1,2-intrastrand GpG (structure a), 1,2-intrastrandApG (structure b), 1,3-intrastrand GpNpG (structure c), and 1,2-interstrand GpG (structure d)

1.7.1.1 Alkylating agents

DNA alkylating agents have been used as anticancer agents by producing significant DNA damage to kill cancer cells [53]. Alkylating agents involve reactions with guanine in DNA though some alkylating drugs exert their strongest effects through alkylation of other bases and other types of cross-links. These drugs add methyl or other alkyl groups onto DNA. This in turn inhibits their correct utilization by base pairing and causes a miscoding of DNA. Alkylating agents can interact via three mechanisms. In the first mechanism, an alkylating agent attaches alkyl groups to DNA bases. This alteration results in the DNA being fragmented by repair enzymes in their attempts to replace the alkylated bases. A second mechanism by which alkylating agents cause DNA damage is the formation of cross-links, bonds between atoms in the DNA. In this process, two bases are linked together by an alkylating agent that has two DNA binding sites. The cross-linking of the two strands of DNA produced by the bifunctional alkylating agent prevents the use of the DNA as a template for further DNA and RNA synthesis leading to inhibition of replication and transcription and, then, to cell death. The third mechanism of action of alkylating agents causes the mispairing of the nucleotides leading to mutations. The alkylating agents, by chemical interactions, form covalent links with DNA. This causes ‘‘mistakes’’ in the DNA that may result in mispairing, substitutions, or excision. The cellular response of these mistakes may inhibit DNA synthesis and proliferation or they may result in apoptosis (process of programmed cell death) [52].

Alkylating drugs are the oldest class of anticancer drugs still commonly used; they play an important role in the treatment of several types of cancers. Most alkylating drugs are monofunctional methylating agents (e.g., temozolomide [TMZ], N-methyl-N’-nitroN-nitrosoguanidine [MNNG], and dacarbazine), bifunctional alkylating agents such as nitrogen mustards (e.g., chlorambucil and cyclophosphamide), [54]. The alkylation of N3 of guanine by (+)-CC1065 (antitumor antibiotic) is shown in Fig. 1.10 [55].

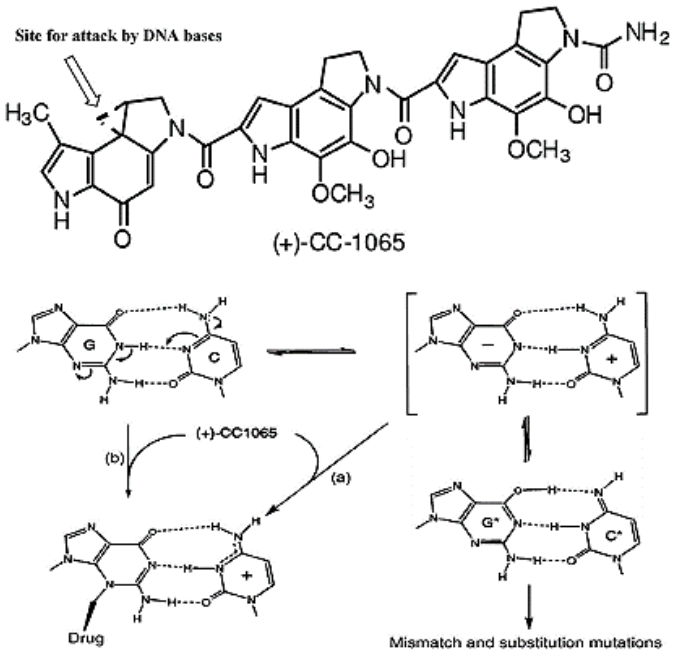

Fig. 1.10: Proposed mechanisms for the alkylation of N3 guanine by (+)-CC1065: (a) tautomerization of the G–C base pair to give the enol-G–imino-C tautomer; (b) a concerted mechanism.

1.7.2 Non-covalent mode of binding

Non-covalent DNA interacting agents, DNA-groove binders and DNA intercalators, are generally considered less cytotoxic than agents producing covalent DNA adducts and other DNA damage. Although the impact of non-covalent compound–DNA interactions on molecular and biochemical pathways is not well characterized, the most important effects include DNA conformational and related structural perturbations, interference with normal DNA protein interactions, such as topoisomerases, as well as effects on mitochondrial DNA function. The non-covalent binding mode is reversible and is typically preferred over covalent adduct formation keeping the drug metabolism and toxic side effects in mind. Non-covalent DNA interacting agents can change DNA conformation, change DNA torsional tension, interrupt protein–DNA interaction, and potentially lead to DNA strand breaks. All of these events can have substantial effects on gene expression [49].

The non-covalent mode of drug–DNA binding is further classified into three types, intercalation, groove binding and external binding (on the outside of the helix)[55].

1.7.2.1. Intercalation

Intercalation of planar organic compounds to DNA was first proposed by Lerman to explain the strong affinity of certain heterocyclic aromatic dyes such as acridines for DNA [56]. Planar heterocyclic compounds act as intercalators which stack between adjacent DNA base pairs leading to significant π-electron overlap.

DNA intercalators are used in chemotherapeutic treatment to inhibit DNA replication in rapidly growing cancer cells. Intercalators are molecules that stack perpendicular to the DNA backbone without forming covalent bonds and without breaking up the hydrogen bonds between the DNA bases. The only known forces that sustain the stability of the DNA–intercalator complex, even more than DNA alone, are van der Waals, hydrogen bonding, hydrophobic, and/or charge transfer forces [57–61]. DNA-intercalator complex is stabilized by π–π stacking interaction and is thus less sensitive to ionic strength relative to the two other binding modes (groove binding and external binding). Structural changes are induced in DNA by intercalators. Intercalation stabilizes, lengthens, stiffens, and unwinds the DNA double helix [62]. For an intercalator to fit between base pairs, the DNA must dynamically open a space between its base pairs by unwinding. The degree of unwinding varies depending on the intercalators. The ethidium cation unwinds DNA by about 26° and proflavine by about 17°. These structural modifications can lead to functional changes, often leading to inhibition of transcription and replication and DNA repair processes, which make intercalators potent mutagens [63,64].

Intercalation is usually independent of the DNA sequence context (a slight GC specificity has been observed). This mode of binding is usually favored by the presence of an extended fused aromatic ligand. With less extended aromatic systems, the intercalation is usually prevented due to interaction of the ancillary ligands with

the phosphodiester backbone, so that only partial intercalation can occur as it is the case for$[Ru(phen)_3]^{2+}$ [65,66](Fig.1.11(A)), whereas Fig.1.11(C) shows the intercalation of Co(II) and Cu(II) complexes into the DNA base pairs [67]. Intercalation of a planar ligand of the complex in the DNA base pairs stack is shown in Fig. 1.11(B) [68, 69].

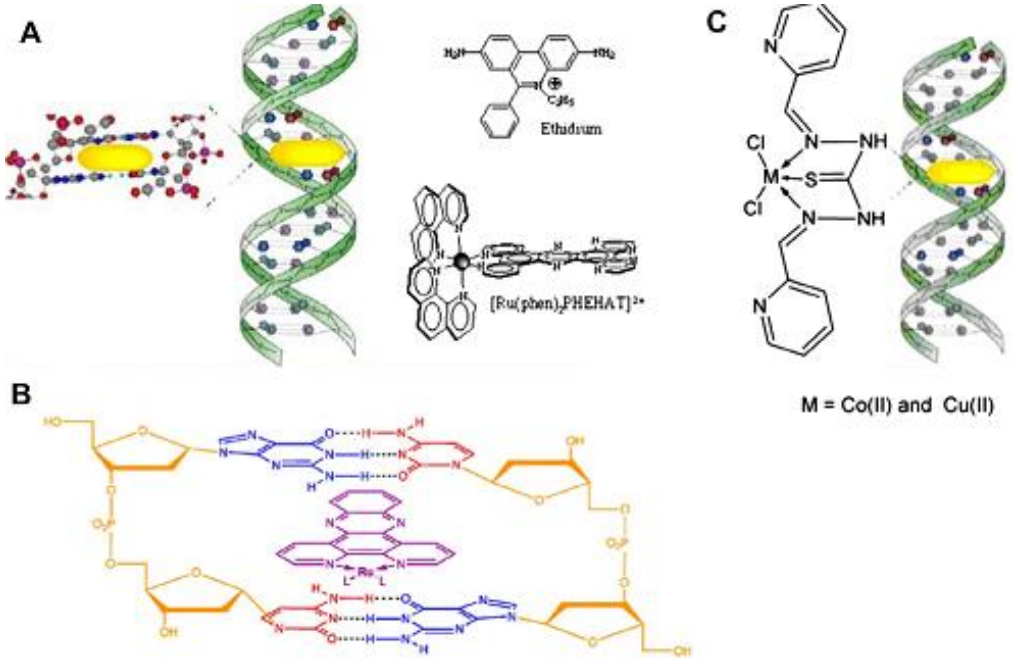

Fig 1.11:(A) Intercalation of a planar ligand of the complex in the DNA base pairs stack. (B) Intercalated ruthenium complex, $[Ru(L)_2(L_0)]^{2+}$(L = bipyridine, 1,10-phenanthroline(phen) and 1,4,5,8-tetraazaphenanthrene (tap) and L_0= dipyridophenazine (dppz) in double-stranded DNA fragment (CG–GC). (C) Intercalation of Co(II) and Cu(II) metalcomplexes of N1,N5-bis[pyridine-2-methylene]-thiocarbohydrazone.

1.7.2.1.1 Modes of intercalation.

There are two major modes of intercalation: classical intercalation and threading intercalation [50,70].

1.7.2.1.1.1 Classical intercalation.

Classical intercalators, such as benzo[a]pyrene (BP) (Fig. 1.12(A)) [71], bind to DNA duplexes with essentially all of their aromatic system inserted between GpG base pairs that form the top and bottom of the intercalation site.

1.7.2.1.1.2 Threading intercalation.

Threading intercalators usually have two side chains on opposite sides of a planar aromatic ring system, the process of adduct formation with DNA is more complicated. In such cases, one of the side-chains must slide through the intercalation cavity in order to form the complex. Favorable interactions of the side-chains with both the major and minor grooves contribute to the adduct stability of the threading intercalators [72]. A threading intercalator occupies and interacts strongly with both the minor and major grooves of DNA simultaneously. For example, the threading intercalation of acridine-4-carboxamides into the duplex 5'-d(CG(5-BrU)ACG)2-3' (Fig. 1.12(B)) [73]. Acridine compounds are able to inhibit topoisomerase I and II enzymes (topoisomerases are essential DNA-targeting enzymes that initially induce a DNA strand cleavage), render DNA damage, disrupt DNA repair and replication, and induce cell death [74].

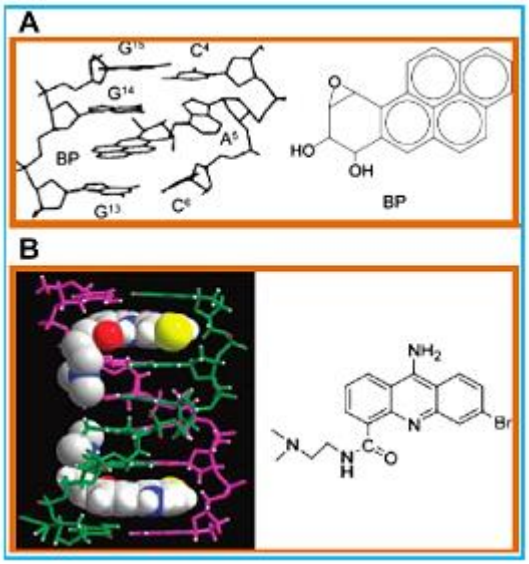

Fig. 1.12: (A) BP group attached to the A5 base intercalates classically between the G13G14 base step of the opposite strand.

(B) X-ray structure showing threading intercalation of 9-amino-6-bromo-DACA (space-filling model) into the DNA duplex 5'-d(C|G(5-BrU)AC|G)2-3' (wireframe).

1.7.2.2 Groove binding

Some small compounds can bind to the minor groove of DNA by vander Walls interaction and hydrogen bonding. Minor groove binding drugs typically have several aromatic rings, such as pyrrole, furan or benzene connected by bonds possessing torsional freedom. Additionally, these drugs can form hydrogen bonds to bases, typically to N3 of adenine and O2 of thymine. The groove-binding molecules are commonly specific to adenine–thymine (AT) rich sequences. This preference in addition to the designed propensity for the electronegative pockets of AT sequences is probably due to better van der Waals contacts between the ligand and groove walls in this region, since AT regions are narrower than GC groove regions and also because of the steric hindrance in the latter, presented by the C2 amino group of the guanine base [75]. Hydrophobic and/or hydrogen bonding are usually important components of this binding process, and provide stabilization.

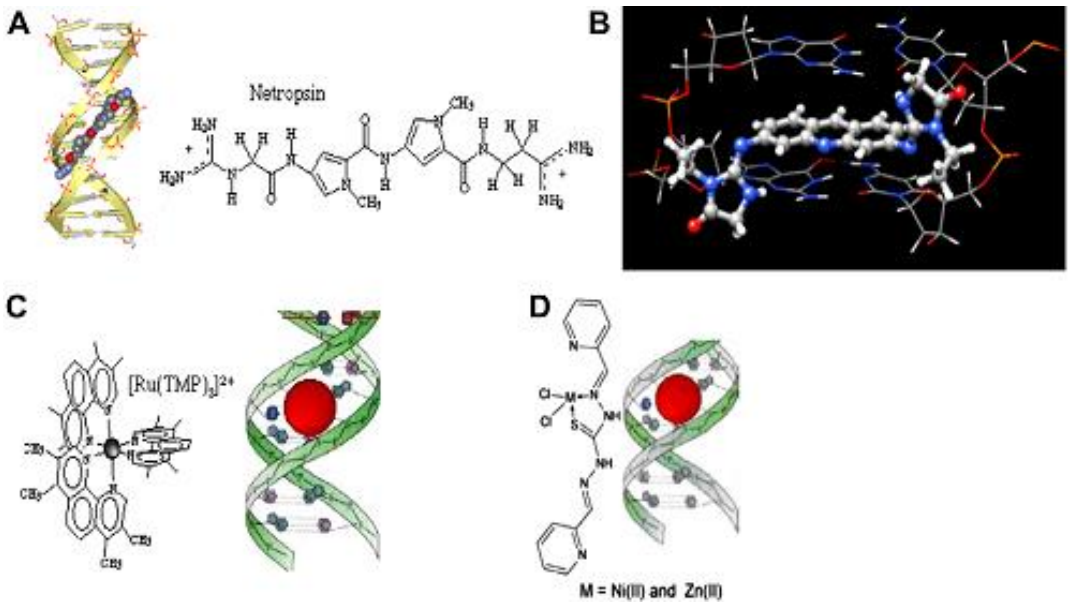

Fig 1.13: (A) DNA complexed with netropsin, a minor groove binder. (B) Putative binding of acridine bis-imidazolidinones (R = ethyl) into adjacent cytosine:guanine base pairs from minor groove side. (C) Adsorption of the $[Ru(TMP)_3]^{2+}$ in the DNA grooves. (D) Groove binding of Ni(II) and Zn(II) metal complexes of N1,N5-bis[pyridine-2-methylene]-thiocarbohydrazone.

The antibiotic netropsin is a model groove-binder (Fig.1.13 (A)). Fig. 1.13(B) shows the generally proposed binding of acridinebis-imidazolidinones (R = ethyl) into adjacent Cytosine: Guanine base pairs from minor groove side [76]. Geometric and steric factors also play a role as shown with $[Ru(TMP)_3]^{2+}$,where the methyl groups prevent intercalation [77](Fig. 1.13(C)). Unlike intercalators, groove-binding drugs induce little or no structural rearrangement of the DNA helix. Fig. 12 D shows the Groove binding of Ni(II) and Zn(II) metal complexes of N^1,N^5-bis[pyridine-2-methylene]-thiocarbohydrazone [68].

1.7.2.3 External binding

This type of binding is electrostatic in nature. Some ligands are also capable of forming non-specific, outside edge stacking interactions with the DNA phosphate backbone. This mode usually occurs where the ligand self-associates to form higher-order aggregates, which may stack on the anionic DNA backbone in order to reduce charge–charge repulsion between ligand molecules. Some metal complexes also interact with DNA through external binding, e.g., binding of Ru(II) complexes which are 2+ positive charge with the DNA phosphate sugar backbone which is negatively charged. This association mode was proposed for $[Ru(bpy)_3]^{2+}$as the luminescence enhancement of this complex upon binding to DNA is strongly dependent on the ionic strength. Cations like Mg^{2+}, usually also interacts in this way (Fig. 1.14) [78].

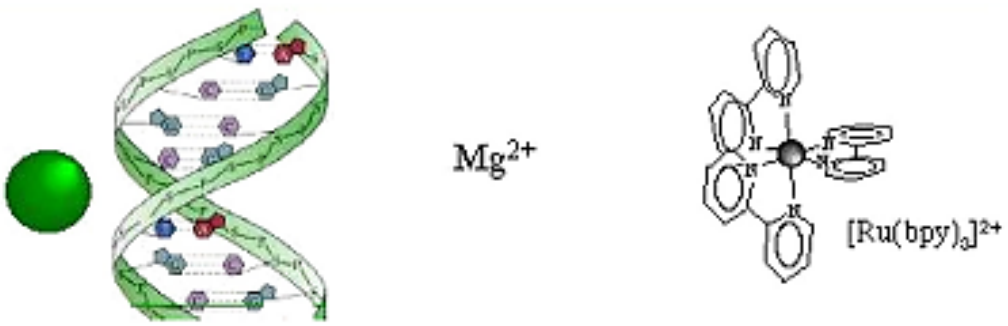

Fig. 1.14: External association of the complex in the atmosphere of ions of the DNA polyelectrolyte.

1.8 DNA cleavage

Cleavage of DNA is a vital process in all living systems. For example, topoisomerase enzymes resolve topological problems of DNA in replication, transcription and other cellular transactions by cleaving one or both strands of the DNA [79]. The restriction enzymes (or restriction endonucleases), protect the cell against virus infection by cleavage of the foreign DNA, or by degrading cellular DNA during apoptosis of the affected cell [80]. The activity of many anticancer drugs rely on their ability to introduce cleavage to the DNA in the (affected) cells [81] (e.g. bleomycin), which can trigger apoptosis [82], leading to the cell death [83]. In general, three different types of DNA cleavage can be distinguished, namely i) DNA hydrolysis, ii) oxidative cleavage and iii) photochemical cleavage, although the last two categories are quite closely related.

1.8.1. Hydrolytic cleavage

Hydrolytic cleavage involves cleavage of phosphodiester bonds on the nucleic acid strands. Hydrolytic cleavage eliminates cytotoxic side effects and resulting fragments are easy to relegate. However the intrinsic stability of the phosphodiester bond makes it one of the most inert chemical functional group for hydrolysis. Usually the DNA fragments produced by restriction endonuclease via hydrolytic cleavage results in 5'-phosphate and 3'-hydroxyl group. The hydrolysis reaction can be simplified as a two-step mechanism. Firstly a nucleophile produced via metal activated water molecules attacks phosphorous atom, forming a penta coordinate intermediate. Secondly the 2'-deoxyribonucleotide fragments with 3'-OH group are removed from phosphorous through scission of P-O bond.

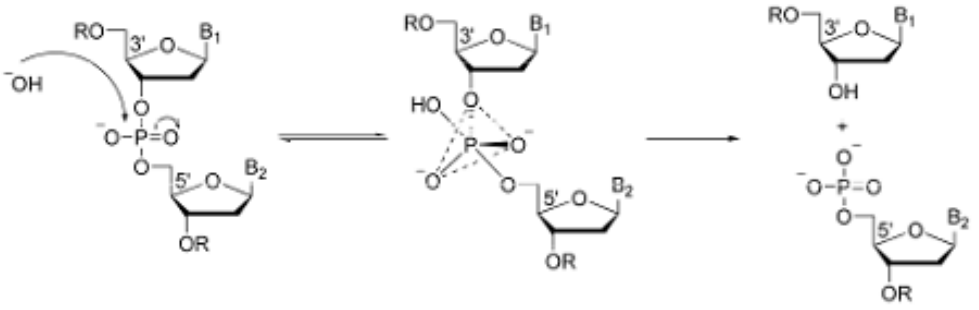

Fig 1.15: Proposed mechanism for the hydrolysis of DNA.

Hydrolysis reactions of nucleic acids mediated by metal ions are important elements in natural enzymatic reactions; which can be exploited in the design of artificial restriction endonucleases. Hydrolysis reactions of the phosphodiester linkage of polynucleotides appear preferable to redox-mediated cleavage reactions, since in the hydrolytic reaction all information is preserved. In redox cleavage by sugar oxidation, for example, both a sugar fragment and free nucleic-acid base are released from the polymer, and, in contrast to hydrolytic chemistry, the direct religation of the fragments becomes practically impossible. Metal ions can be effective in promoting hydrolysis of the phosphodiester, since they can function as Lewis acids, polarizing the phosphorus-oxygen bond to facilitate bond breakage, and can also deliver the coordinated nucleophile to form the pentacoordinate phosphate intermediate. Fig.1.15 illustrates one crystallographically characterized model system developed by Sargeso and coworkers, where hydrolysis of a model phosphodiester was enhanced dramatically by taking advantage of both the acidic and the nucleophilic characteristics of the bound cobalt (III) species [84](Fig 1.16).

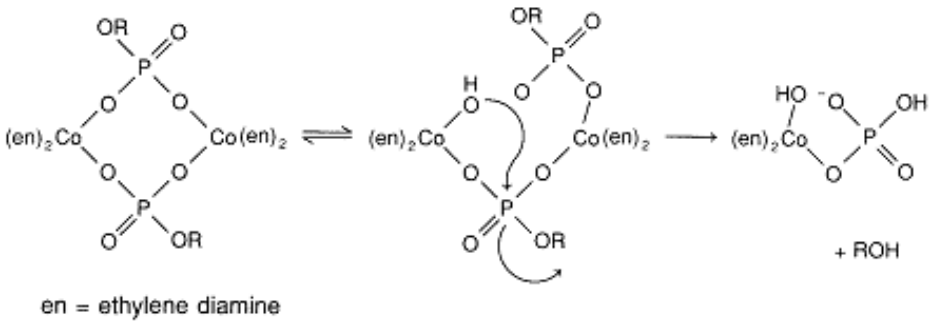

Fig. 1.16: Hydrolysis reactions catalyzed by metal ions and complexes. Illustration of a phosphate ester hydrolysis in a binuclear model complex catalyzed by coordinated cobaltic ions, with one metal ion functioning as a lewis acid and the other functioning to deliver the coordinated hydroxide.

1.8.2. Oxidative cleavage

This method of cleavage involves the oxidation of deoxy ribose by abstraction of sugar hydrogen or oxidation of nucleobases. Oxidative cleavage is usually mediated by the presence of additives and photo induced DNA cleaving agents i.e. an external agent like light or H_2O_2 is required to initiate cleavage. In this method the DNA fragments cannot be religated. Oxidative cleavage can occur both at the carbohydrate level and at the nucleic base level and can result in the damage of all four nucleobases or the deoxy ribose sugar. Generally hydroxyl radical species $OH^{\cdot}$ is involved in this oxidative cleavage. The mechanism of oxidative cleavage occurs in 3 ways: hydrogen abstraction, addition and electron transfer. If the oxidative cleavage occurs at the carbohydrate, abstraction of one hydrogen of deoxyribose can initiate

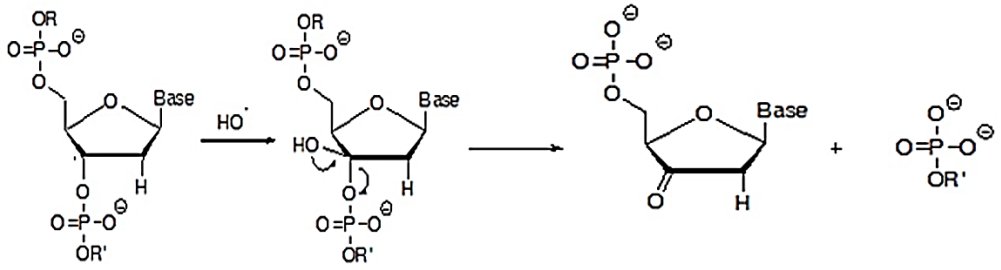

Fig 1.17: Cleavage at nucleobases.

the oxidative cleavage process. The process following the C-3' abstraction of deoxyribose is shown in Fig 1.17. The oxidation at the nucleic base level occurs preferably at guanine because it's lower oxidation potential. Hydroxyl radical reacts with the heterocyclic bases in DNA by addition. In pyramidines OH adds to the C5 or C6 double bond leading to cleavage. In purines the hydroxyl ion binds to the C4, C5 & C8 [85].

The application of Fenton chemistry to promote site-specific or sequence neutral cleavage of DNA was first demonstrated by Dervan and coworkers [86], (Fig. 1.18) and has provided the basis for the design of a tremendous range of new and valuable DNA cleavage agents. Other metal ions such as Cu(Il) can also promote redox-mediated cleavage of DNA through reactions on the sugar ring.

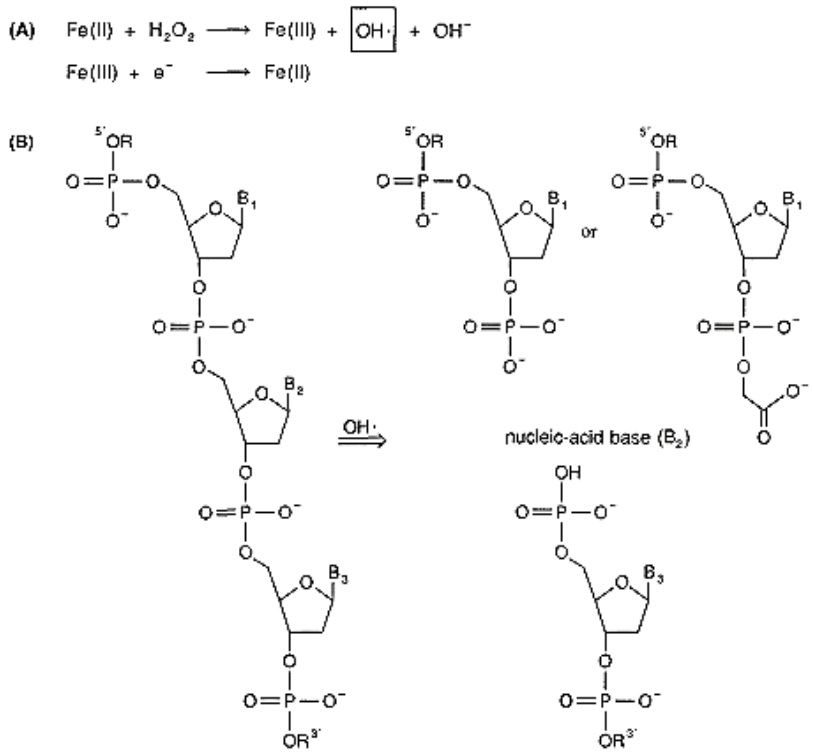

Fig 1.18: An illustration of DNA strand cleavage mediated by hydroxyl radicals by Fentons reaction (A) of $Fe(EDTA)^{2-}$ with hydrogen peroxide. The cleavage scheme (B) shows the products obtained as a result of initial C4'-H abstraction by hydroxyl radicals.

1.8.3. Photoinduced DNA Cleavage

Photocleavage of nucleic acids allows the use of light to trigger nuclease activity. Nucleases that are activated by visible or near-UV light can be used for examination of processes such as transcription and to probe nucleic acid structure as photo foot printing and photo-sequencing agents. On the other hand, photosensitization of DNA by drugs may be useful as a potential anti-tumor therapy. DNA photo cleavage can occur by a wide variety of mechanisms such as hydrogen atom abstraction from the sugar ring by photo chemically generated radicals [87], direct electron transfer from the base (usually guanine) to the photo excited cleaver [88], singlet oxygen production by transfer of energy from the excited photo cleaver, and [89] formation of base adducts.

DNA damage initiated by photosensitization can be divided in two major types; a one electron process (Type I)and a pathway involving singlet oxygen (Type II) [90,91].In the first type (Type I process), the cleaving agent is excited and generates sequentially a superoxide radical from molecular oxygen via an electron transfer step. Superoxide itself is a rather poor oxidant, and it can be further reduced (leading to H_2O_2 and OH) or it can function as a reductant. The DNA damage observed via this pathway is mainly guanine oxidation, formed via guanine radical cations [90,91]. This results in the formation of base labile sites in the DNA. In a Type II process, the photo excited compound generates singlet oxygen, which only modifies guanine residues, in contrast to superoxide.

1.9 Review of Literature

The first review regarding the interactions of metal ions with quinolone was published tewelve years ago and discussed selected crystal structures of quinolone–metal compounds, different physico-chemical methods of characterization, as well as some results of bioactivity test [92]. The structural characteristics of a part of fluoroquinolone complexes and their biological activity were reviewed few years ago [93]. A comprehensive review by G. Psomas *et al* [94] presented the structures and the biological activity of complexes of some quinolones with Cu(II), Ni(II), Co(II) and Zn(II) and analysed the influence of the second ligand on biological activity. Valentina Uivarosi [95] published an updated review on metal complexes of quinolone antibiotics and their applications. The author discussed the interactions of quinolones with metal ions and their important consequences for the solubility, pharamakokinetic and bioavailability of quinolones as well as in the mechanism of action of these bactericidal agents. New strategies in the design of metal complexes of quinolones that have led to compounds with anticancer activity was presented. Analytical applications of metal quinolone complexes for determination of quinolones or metal ions was mentioned.

Though a large number of studies have been reveiwed in the literature regarding the interactions between various quinolone derivatives and metal ions, however, a thorough survey of literature on antitumor activities of metal-fluoroquinolates has revealed only a limited number of studies. Li *et al* [96] have examined the anti-proliferative activity of the ternary copper complexes of ofloxacin and levofloxacin with 1,10-phenanthroline as ancillary ligand against the leukemia HL-60 as well as liver cancer HepG2 cell lines, where the levofloxacin–copper complex was found to be more potent than the corresponding ofloxacin compound. Similarly, the mixed-ligand copper complexes of norfloxacin and bipyridyl/ 1,10-phenanthroline as ancillary ligands were found to be more active than the free ligand against HL-60 (human acute myeloid leukemia) and K562 (human chronic myeloid leukemia) cell

lines, respectively. W.H. Mahmoud *et al* [97] have reported antitumor activity of lomefloxacin metal complexes, ([Co(LFX)(H_2O)$_4$]·Cl_2 and [Zn(LFX)(H_2O)4]·Cl_2) against the breast cancer cell line MCF7. These complexes were found to be more active than the corresponding lomefloxacin.

Padhye et al. described a neutral dimeric copper complex of sparfloxacin and its phenanthroline derivative which showed considerable enhancement in its anti-proliferative activity against hormone independent BT20 breast cancer cells [98]. which is interesting since high expression of topoisomerase-II has been shown to be correlated with the hormone-independent pathway. Recently they have reported novel ternary moxifloxacinato–copper(II) complexes with N-containing ligands screened for anti-proliferative and apoptosis-inducing activity against multiple human breast cancer cell lines (hormone-dependent MCF-7 and T47D as well as hormone-independent MDA-MB-231 and BT-20) [99]. The results indicated that the parent compound moxifloxacin does not exert any inhibitory activity against breast cancer cell lines examined. On the other hand, the copper conjugate and its nitrogen adducts exerted growth inhibitory and apoptosis-inducing activity against breast cancer cell lines without any substantial effect on non-tumorigenic breast epithelial cells MCF-10A at equimolar concentration, suggesting a cancer cell-specific activity. Additionally, complexation with copper has been reported to inhibit the efflux mechanism effectively, thereby leading to enhanced intracellular accumulation of the quinolone drugs as reported by Jakics et al.

Letícia R. Teixeira et al. have reported gold (III) complexes with general formula [$AuCl_2$L]Cl (L = norfloxacin, levofloxacin, sparfloxacin) which were tested against A20 (murine lymphoma), B16-F10 (murine melanoma) and K562 (human myeloid leukemia) tumor cell lines [100]. Results indicate that the free ligands did not show significant activity in the tumor or normal cell lines, whereas the complexes are more active than the parent drugs. Turel *et al.* have reported anticancer activity of organometallic ruthenium complexes chlorido(η6-p-cymene)(nalidixicato-

κ2O,O)ruthenium(II) and chloride (η6 cymene)(cinoxacinato-κ2O,O) ruthenium(II) in human A549 (nonsmall cell lung carcinoma), CH1 (ovarian carcinoma), and SW480 (colon carcinoma) cells. The compounds were shown to be non-cytotoxic to the various cell lines [101].

Recent research has focused on increasing the antitumor activity of polyoxometalates (POMs) by introduction of drug molecules into the POM surface, and such molecules could be quinolone chelates. Jingmei Lu et al. have reported antitumor activity of [{[Co(PPA)$_2$]H$_2$[SiW$_{12}$O$_{40}$]}·HPPA·3H$_2$O] on MCF-7 cell lines. The MTT investigations found that{[Co(PPA)$_2$]H$_2$[SiW$_{12}$O$_{40}$]}·HPPA·3H$_2$O] have shown higher antitumor activity than the parent compound SiW$_{12}$ [102]. This superiority in the antitumor activity could be explained from the synergism of POMs and Co-PPA. This work implies that the introduction of M-PPA/HPPA into the polyoxoanion surface could increase their antitumor activity and make the compounds to penetrate into the cells easily.

Jing-Quan Sha et al. reported antitumor activity of three complexes of pipemidic acid, [Cu(PPA)$_2$]$_2$·[PW$_{12}$O$_{40}$]·6H$_2$O),[HPPA]5·[PW$_{11}$CdO$_{39}$]·2H$_2$O and [HPPA]3·[PW$_{12}$O$_{40}$]·2H$_2$O against PC-3, Hela and HepG2 cells [103]. The results indicated that these three new compounds possess stronger antitumor activity than the parent compound (PW$_{12}$O4$_0$), which may be due to changes of their properties. The results show that introduction of TM-PPA/PPA into the polyoxoanion surface could increase their antitumor activity and make the compounds penetrate into the cells easily. Furthermore, the antitumor activity of the compounds can be modulated by their different structures.

SUMMARY OF PRESENT WORK

The present investigation focuses on the structural modification responsible for the transformation of an antibacterial to anticancer agents. A distinctive feature of drugs based on the quinolone structure is their remarkable ability to target topoisomerase II enzymes, which is a marker of cell proliferation in normal as well as cancerous tissue. Hence, these cytotoxic quinolones represents an exploitable source of new anticancer agents. Their ability to bind metal ion cofactors represents an additional means of modulating their pharmacological activity. As a result the quinolone agents link antibacterial and anticancer chemotherapy together and provides an opportunity to clarify drug mechanism across divergent species.

Chapter 1

The chapter presents a general introduction of quinolones, with special mention of metal – quinolonates, DNA interactions and neoplastic activity.

Chapter 2

This chapter presents a general introduction of material and methods.

Chapter 3

We have explored the DNA binding, DNA cleavage and antiproliferative activity of transition metal complexes of fluoroquinolones. All the synthesized complexes are good binders to DNA. Viscometric titration data supports the intercalative mode of binding of complexes to CT-DNA. The present work clearly demonstrates that there is enhancement in the antiproliferative activity of fluroquinolones on complexation with metal ions.The synthesized complexes induce apoptosis.

Chapter 4

Nitrogen adducts of copper &vanadyl with fluroquinolones (GFL/MFL) are endowed with antiproliferative activity against A549 cells. The synthesized complexes strongly intercalates to DNA and induces apoptosis which could be of application in target based cancer therapy.

Chapter 5

Mixed ligand copper complexes of moxifloxacin with mannich bases of ferrocene have been synthesized and characterized. Incorporation of ferrocene moiety results in significant structural change in the ferrocenyl complexes compared to non-ferrocenyl analogues which controls their biological activity. The complexes are groove binders to CT-DNA. The complexes predominantly internalize in the A549 cells and induces apoptosis.

Chapter 6

Metal complexes of moxifloxacin with curcumin as ancillary ligand have been synthesized. The complexes significantly induces ROS generation and apoptosis. It was found that all the complexes activate Caspases, which in turn cleaves PARP and thus causes DNA damage and apoptosis.

Chapter 7

In the present study, ternary Cu(II) complexes with isatin hydrazones and MFL were synthesized which demonstrated to possess higher antiglycation activity than isatin Schiff bases, when tested by glucose-BSA assay in a dose-dependent manner. The mechanism of antiglycation activity of complexes will be the subject of future studies.

Chapter 8

A series of mixed ligand Zinc(II) and VO(II) complexes with dipeptides and moxifloxacin ligand have been synthesized. Vanadyl(II) complexes act as novel sensors for selective sensing of Cu^{2+}.

The Zn(II) complexes produce a detectable level of luminescence in aqueous solution in the absence of DNA and display substantial enhancement in luminescence on DNA binding. These examples show that the luminescent metal complexes can be utilized to exploit the specific interactions between nucleic acids and metal ions for the construction of label-free DNA sensors.

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