MedChemComm

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: J. V. Mehta, S. B. Gajera, D. Raval, V. R. Thakkar and M. N. Patel, *Med. Chem. Commun.*, 2016, DOI: 10.1039/C6MD00251J.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/medchemcomm

View Article Online
DOI: 10.1039/C6MD00251J

MedChemComm

PAPER

Biological assessment of substituted quinoline based heteroleptic organometallic compounds†

Jugal V. Mehta,[a] Sanjay B. Gajera,[a] Dilip B. Raval,[b] Vasudev R. Thakkar[b] and Mohan N. Patel*[a]

A series of substituted quinoline derivatives were synthesized via the Friedländer condensation reaction using substituted 2-aminobenzophenon with a 2-acetyl pyridine or 2-acetyl thiophene in presence of sodium methoxide. A series of substituted quinoline based organometallic ruthenium complexes of the type [(Cp*)Ru(L^n)Cl]• Cl (where, Cp*= pentamethylcyclopentadienyl ring, L^n=L^{1-7}= quinoline ligands) were synthesized and characterized by elemental analysis, electronic spectra, conductance measurements, thermogravimetric analysis (TGA), Fourier transform infrared (FT-IR) and mass spectrometry. By analogy with structure to a piano stool these are known as piano stool complexes, in which metal centre being coordinated by pentamethylcyclopentadienyl ring, chlorido ligand and chelating quinoline ligand. All compounds were studied for their *in vitro* antimicrobial activity against five unlike bacterial strain. All compounds were investigated for their interaction with Herring Sperm (HS) DNA, developing absorption titration (K_b=0.34–6.25×10^5 L mol^{-1}) and viscosity measurements. The studies conclude the classical intercalative mode of DNA binding. The DNA-binding property of all the compounds were also examined theoretically using molecular docking study and it prove intercalation binding mode between compounds and nucleotide base pairs of HS DNA. The capability of the compounds to cleave pUC19 DNA was examined by chemical nuclease activity. The results indicate that the ruthenium complexes effectively promote the cleavage of plasmid DNA than that of respective quinoline ligands. All compounds were evaluated for cytotoxicity activity against *S. pombe* cells at a cellular level and exhibit enhanced activity against *S. pombe* cells compared to the quinoline ligands. All synthesized compounds were also evaluated for their *in vitro* antimalarial activity [50% Inhibition concentration (IC_{50}) = 0.55–1.84 mg/L] against *Plasmodium falciparum* strain as well as *in vitro* cytotoxic activity [50% Lethal concentration (LC_{50}) = 5.64–119.67 mg/L] against brine shrimp (Artemia cysts) eggs.

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

1. Introduction:

In view of the environmental, practical and economic issues, the development of a clean procedure for the preparation of heterocyclic compounds is a major task in modern heterocyclic chemistry. Heterocyclic ring containing compounds have concerned significant interest owing to their immense biological activities in the field of bioorganic and biomedical compounds, pharmaceuticals and others. Nitrogen-containing heterocycles are a very important group of organic compounds because of their wide application in medicine, agriculture, and technology. Among these, quinoline derivatives are of significant synthetic interest due to their diverse range of biological activities. In the recent time, quinoline nucleus has gathered an immense attention among chemists as well as biologists as it is one of the key building elements for many naturally occurring compounds. Compounds containing a quinoline framework have been found applications as pharmaceuticals and being general synthetic building blocks.[1] Industrial, biological, and synthetic significance places this scaffold in a significant position. Therefore, significant effort continues to be directed toward the development of new quinolines. In particular, there is much current interest in the quinoline ring system especially in the area of medicinal chemistry, and moreover it is a ubiquitous sub-structure found in many biologically active natural products.[2-4] In this regard, quinolines have occupied a unique position in the design and synthesis of novel biologically active compounds since they are often used as anti-inflammatory, anti-asthmatic, anti-tuberculosis, anti-bacterial, anti-hypertensive, anti-tumor and anti-malarial agents.[5]

The complexes as drug work in one of the two ways: some use a process called redox chemistry to take electrons from the bonds holding the target molecules together. Others use hydrolysis, meaning that they breakdown the target's chemical waterproofing, so that the water that is naturally present in a cell react with the target. The field of inorganic chemistry in medicine may usefully be divided into two main categories: firstly, metal-based drugs where central metal ion is usually the key feature of the mechanism and secondly, ligands as drugs which target metal ions in some form, whether free or protein-bound. Metals can play an important role in modifying the pharmacological properties of known drugs after

†The authors declare no competing interests.

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar–388 120, Gujarat, India. E-mail: jeenen@gmail.com

Electronic Supplementary Information (ESI) available: See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C6MD00251J

coordinating to a metal. Chelation causes drastic change in biological properties of ligands as well as metal moiety and in many cases it causes synergistic effect of metal ion and ligand both. For example, complexation of nonsteroidal anti-inflammatory drugs to copper overcomes some of the gastric side effects of these drugs.[6] Metal based drugs play an important part in the history of medicinal chemistry. A variety of transition metal complexes with different metal centres and ligands of diverse structures were synthesized and studied for their biological activity. Because of the range of coordination geometries, metal complexes provide more stereochemical variability than is possible in organic molecules and often introduce new elements of chirality which may be important for biological molecule recognition and interaction. The metal complexes can also be extremely positively-charged: since many biological structures, such as several types of phospholipids, DNA and RNA, and some regions of proteins – are negatively charged, for electrostatic reasons the positive charge of metal complexes could aid the binding with intracellular targets.

Among the transition metal complexes or coordination compounds, ruthenium-based complexes have been widely studied and some have displayed significant biological activity. This can be due to their ability to strongly bind nucleic acids and proteins, ligand exchange kinetics similar to those of their platinum counterparts. Ruthenium based organometallic compounds are progressively gaining in importance as promising aspirants for the design of novel and more effective metal-based drugs.[7-11] Also various metal-containing compounds, ruthenium compounds are considered stable under both highly acidic and alkaline conditions.[12] Applications of ruthenium compounds have a long history. This includes antimicrobial activity, anti-cancer activity, DNA binding, and DNA cleavage. It is well known that, deoxyribonucleic acid (DNA) is an important target in the organism for some metal based drugs or reagents. These reagents can interact with DNA thereby changing the replication of DNA and inhibiting the growth of the tumor cells.[13] Therefore, considerable attentions have focused on the design and synthesis of new complexes, especially transition metal complexes, which have enhanced selectivity and novel modes of DNA interaction, such as non-covalent interactions that mimic the mode of interaction of proteins with DNA.[14] It has been reported that the metal complexes can interact with DNA non-covalently in the mode of intercalation, groove binding and electrostatic effect.[15, 16] The effectiveness mainly depends on the mode and affinity of the binding between the complexes and DNA.[17] The insertion of biologically-active ligands into metal coordination compounds deals much scope for the design of novel drugs with improved and targeted activity. Studies on such complexes indicate that new mechanisms of action are favorable when combining the bioactivity of the ligand with the properties inherent to the metal, leading to the possibility of overcoming current drug resistance pathways.

The antitumor activity of many organometallic ruthenium complexes is generally related to their enhanced DNA binding affinity, which involves covalent coordination or simultaneous intercalation of extended aromatic groups and specific hydrogen bonding depending on the particular type of ligands used. In this regard, substituted quinoline ligands were synthesized and combined with the ruthenium(III)-pentamethylcyclopentadienyl ring moiety to enhance their DNA-binding properties, so as to achieve different biological functions and to maximize their effectiveness as therapeutic agents. Here, we used pyridine/thiophene substituted quinoline ligands. Because pyridine is found to have a large number of biological activities such as antiviral, anticancer, antimicrobial, antidiabetic, antitubercular etc. Pyridine is also found in the form of vitamin B3 i.e pyridoxine.18 In medicinal chemistry, thiophene derivatives have been very well known for their therapeutic applications. Many thiophene derivatives have been developed as chemotherapeutic agents and are widely used. Thiophene nucleus is one of the most important heterocycles exhibiting remarkable pharmacological activities.19 Also we used halogen substituted quinoline ligands because halogens are more electronegative atoms which improves its biological activities.

This article mainly focuses on exploring the trend in DNA-binding affinities of synthesized compounds and important differences in some related properties. Understanding the features that contribute to recognition of DNA by metal based drugs or small molecules is crucial for the enhancement of drugs targeted at DNA. In present study, a series of substituted quinoline derivatives were synthesized via the Friedländer condensation as well as a series of piano-stool ruthenium coordination compounds were synthesized and well characterized. All synthesized compounds were evaluated for their biological applications. In assessment of these inspections and in persistence of the research work on bioactive heterocycles, it was anticipated to plan and viewing them for biological applications.

2. Materials and methods

Details of materials, reagents and physical measurements are shown in supplementary material 1.

2.1. General method for synthesis of quinoline ligands (3a-3g)

Detail process for the synthesis andof quinoline ligands is shown in supplementary material 1. The proposed reaction for the synthesis of ligands (3a-3g) is shown in scheme 1. ^{1}H NMR and ^{13}C NMR spectra of ligands are shown in supplementary material 2 and 3, respectively. Detail characterisation of ligands are shown in supplementary material 1. The possible mechanism for 4-phenyl-2-(pyridin-2-yl) quinoline (3a) via Friedländer condensation reaction is proposed in scheme 2.

2.2. General synthesis of the complexes

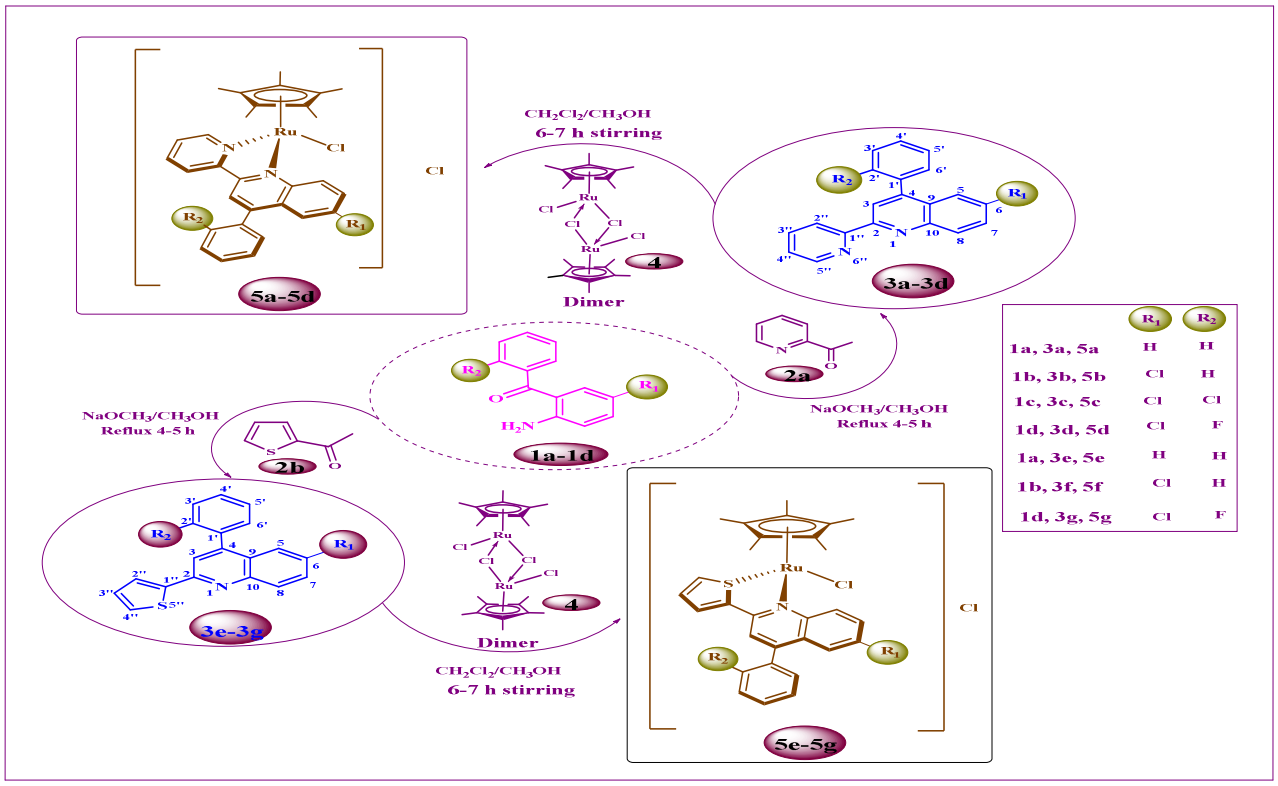
An organometallic half-sandwich Ru(III) metal complexes **(5a-5g)** of the general formula $[(Cp^*)Ru(L^n)Cl]\bullet Cl$ were synthesized by the reactions of $[\{(Cp^*)Ru(\mu\text{-}Cl)Cl\}$ with the respective ligands **(3a-3g)** in a 1:2 molar ratio of dichloromethane and methanol, respectively. Detail synthesis and characterisation of complexes are shown in supplementary material 1.

2.3 Biological applications of synthesized compounds

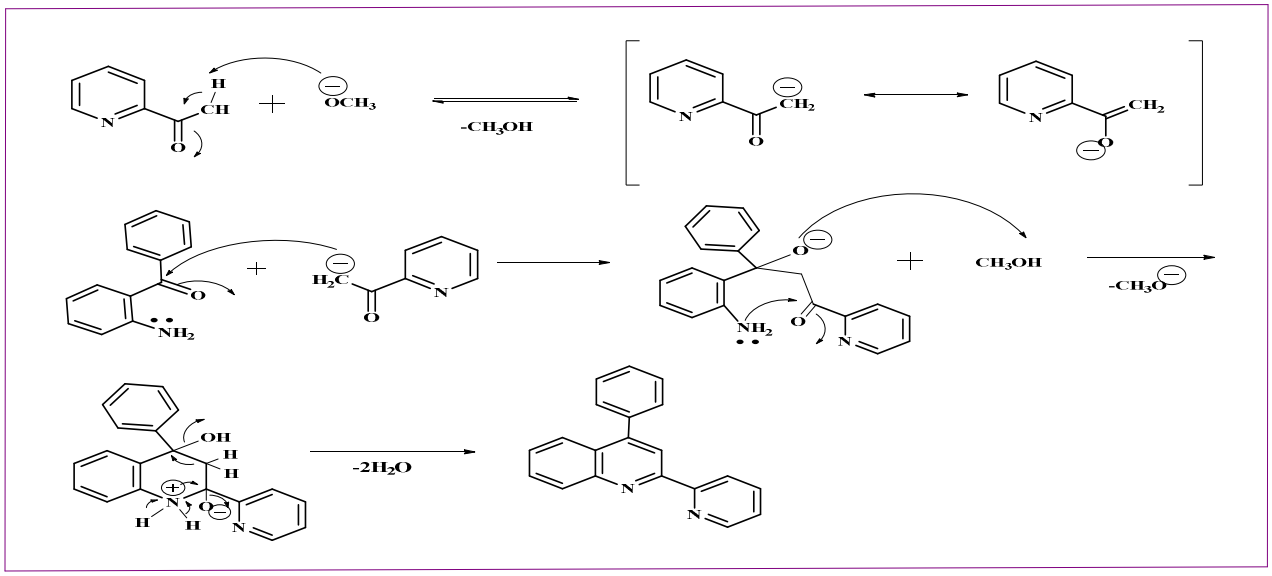
View Article Online
DOI: 10.1039/C6MD00251J

All experimental part of biological studies are included in supplementary material 1.

View Article Online
DOI: 10.1039/C6MD00251J



Scheme 1. Synthesis of quinoline derivatives **(3a–3g)** and its complexation with ruthenium metal ion.



Scheme 2. The possible mechanism of formation 4-phenyl-2-(pyridin-2-yl) quinoline via Friedländer condensation reaction.

3. Results and discussion

3.1 Magnetic moments, electronic spectra analysis and conductance measurements

The room temperature magnetic moments show that the organometallic Ru(III) complexes are paramagnetic (2.15–2.23 BM), which corresponds to the +3 oxidation state of ruthenium. The μ_{eff} values indicate a single unpaired (S = ½)

View Article Online
DOI: 10.1039/C6MD00251J

electron and are consistent with low-spin t_{2g}^5 configuration for ruthenium(III) ion in all complexes.[20]

The electronic spectra of synthesized complexes are recorded using DMSO as a reference. The ground state of Ru(III) (t_{2g}^5 configuration) is $^2T_{2g}$.[21] In most of the Ru(III) complexes, the electronic spectra show only charge transfer bands.[22] All Ru(III) complexes exhibit three dissimilar bands, one band ranging from 358–370 nm, which is attributed to metal to ligand charge transfer (MLCT) transition. A high energy two bands, ranging from approximately 345–351 nm and 285–292 nm in the ultraviolet region are attributed to the LMCT transition (n–π*) and intra-ligand charge transfer (π–π*) transitions for the quinoline ligands, respectively. The pattern of the electronic spectra of all the organometallic Ru(III) complexes indicate the existence of piano-stool environment around the ruthenium(III) metal ion.[23, 24]

The molar conductance values for the synthesized low spin Ru(III) complexes were determined in methanol and are found in the range of 39–67 $cm^2\ \Omega^{-1}\ mol^{-1}$, indicating electrolytic nature and presence of one counter ion outside the coordination sphere of Ru(III) complexes. So, we conclude that all synthesized organometallic Ru(III) complexes are ionic in nature.

3.2 LC–MS analysis

The LC–MS spectrum and probable mass fragmentation pattern of complex (5a) are shown in supplementary material 4 and 5, respectively. Mass spectrum of the complex (5a) shows molecular ion peak $[M-Cl]^+$ and $[M-Cl+2]^+$ at 554 m/z and 556 m/z, respectively due to presence of one chlorine atom. The peak at 519 m/z is due to loss of one chlorine atom. The peak at 384 m/z is due to loss of pentamethylcyclopentadienyl ring. The peak at 237 m/z is due to loss of quinoline ligand. The peak at 282 m/z corresponds to quinoline moiety. The peak at 135 m/z corresponds to pentamethylcyclopentadienyl ring.

3.3 Thermo gravimetric (TG) studies

Thermo gravimetric analysis determines the weight changes of a sample, as a function of temperature. Weight changes as a function of temperature indicate decomposition of material. The temperature, at which no weight loss takes place, reveals the stability of the material. Another important piece of information that can be obtained by TGA is the amount of weight lost by heating the sample to a given temperature. This information helps the chemist to determine the composition of a compound and to follow the reactions involved in its decomposition.

The characteristic thermo gravimetric plot (mass loss % to temperature in °C) of complex (5a) shows three distinct mass losses.[25] 1st mass loss occurs between 40–130 °C, 2nd between 180–240 °C, and last between 260–650 °C. Mass loss occurring during 1st decomposition step corresponds to lose of one chlorine atom (6%), whereas mass loss during 2nd step relates to decomposition of other chlorine atom which bind by one covalent bond to metal center (6%), and 3rd step relates to decomposition of the Cp* and quinoline ligand (71%), leaving behind metal oxide as residue.

3.4. Biological applications of synthesized compounds

3.4.1 Cellular level bioassay using *S. pombe* cells

Concentration(mg/L)	2	4	6	8	10
Compounds	% Viability				
3a	77±2	74±2	69±2	67±2	62±2
3b	75±2	72±2	68±2	66±2	61±1
3c	72±2	70±2	66±2	63±1	59±1
3d	70±1	69±2	65±2	61±1	57±1
3e	82±3	79±2	76±2	73±3	70±2
3f	80±2	77±2	75±2	72±2	68±2
3g	79±2	76±5	72±2	68±2	65±1
5a	65±2	63±2	60±1	56±1	48±1
5b	63±1	61±1	58±1	53±1	46±1
5c	60±1	57±1	55±1	50±1	45±1
5d	59±1	56±1	53±1	49±1	44±1
5e	73±1	70±2	65±2	62±2	52±1
5f	69±1	67±1	63±1	60±1	51±1
5g	68±1	66±1	62±1	58±1	50±1
DMSO			96±3		
Untreated cells			97±3		

Table 1: Effect of compounds on viability of *S. Pombe* cells at different concentrations with error uncertainty in the value ±5%

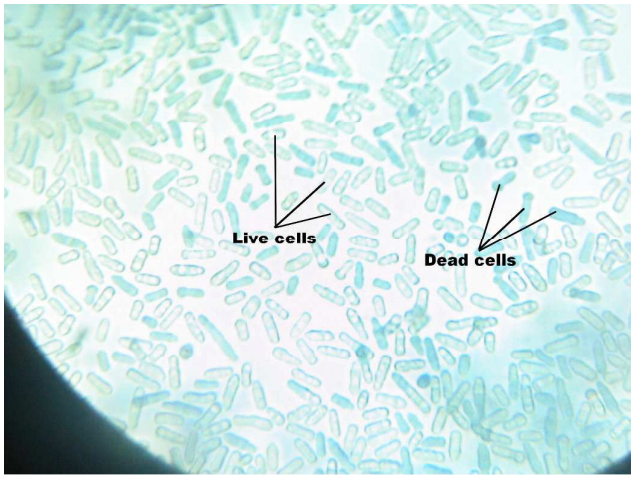


Figure 1. Effect of compounds on *S. pombe* cells. Dead cells are seen dark whereas live cells are seen transparent.

The cellular level study of free quinoline ligands (3a-3g) and synthesized organometallic Ru(III) complexes (5a-5g) was tested against *S. pombe* cells in terms of the percentage viability. Table 1 shows that all the complexes are active against *S. pombe* cells than those of respective quinoline ligands. *S. pombe* cells has become an important tool to study

View Article Online
DOI: 10.1039/C6MD00251J

cell biology due to its eukaryotic and fairly big size characteristics. Cell death caused by toxicity of the chemically synthesized compounds could be easily observed by vital staining which is shown in figure 1. The toxicity is found to vary with the type of substituent present and concentrations of the synthesized compounds. General observation is that the cytotoxicity is increases with concentration of compounds increases. After the treatment, many of the *S. pombe* cells are killed due to toxic nature of the compounds. Complex (5d) is the most active amongst all the compounds. All the complexes show comparable study to $[Ru^{III}(L^{1-7})_3]\bullet(PF_6)_3$ compounds reported in published literature.[11]

3.4.2 *In vitro* brine shrimp lethality bioassay (BSLB)

The brine shrimp lethality bioassay is considered as a useful method for preliminary calculation of toxicity of compounds and development in the assay procedure of bioactive compound, which indicates cytotoxicity as well as a wide range of pharmacological activities of the compounds. All the synthesized compounds were studied for their cytotoxicity using the protocol of Meyer et al.[26] The method is inexpensive, rapid, reliable and economical. Results for the lethality are noted in terms of deaths of larvae. The mortality rate of brine shrimp nauplii is found to increase with increasing the concentration of compounds. A plot of the log of sample's concentration versus percentage of mortality showed a linear correlation. From the graph, the LC_{50} values of the compounds are calculated, and they are found in the range of 5.64–119.67 mg/L (supplementary material 6). All the complexes show superior cytotoxicity than $[Ru^{III}(OFL)(PPh_3)_2Cl_2]$,[27] $[Ru^{III}(PFL)(PPh_3)_2Cl_2]$,[27] $[Ru^{III}(L^{4-6})PPh_3)Cl_3]$[28] and comparable to $[Ru^{III}(L^{1-7})_3]\bullet(PF_6)_3$ compounds reported in published literature.[11]

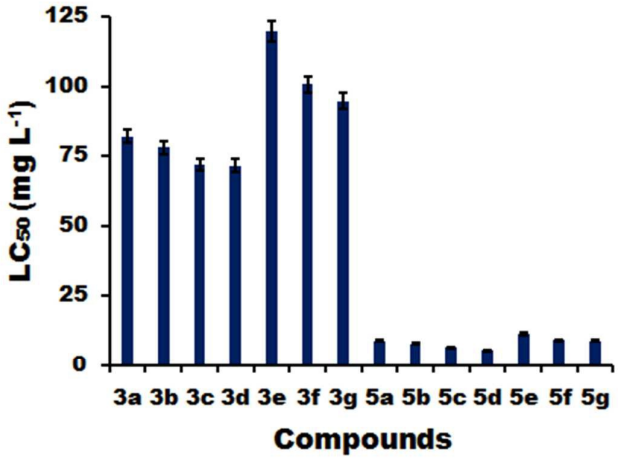


Figure 2. Plot of LC_{50} values of different compounds in mg/L using Brine shrimp. Error bars represent standard deviation of three replicates.

From the data recorded, complex (5d) is the most potent amongst all the compounds. Figure 2 shows that the synthesized complexes are good cytotoxic agent than that of respective ligands. The degree of lethality is found to be directly proportional to the concentration of the compounds. The tested complexes have strong cytotoxic activity but this examination is a primary one and further tests are required to investigate its actual mechanism of cytotoxicity and its probable effects on higher animal model and on cancer cell line. It recommends that the complexes can be used as potent cytotoxic agents with the hope of adding arsenal of weapons used against the fatal disease cancer.

3.4.3 *In vitro* antimicrobial study

A relative study of *in vitro* antimicrobial screening values of the quinoline ligands and their complexes indicates that the metal complexes show superior activity against five unlike microorganisms when incorporated to the ligands. The minimum concentrations of compounds that induced a complete growth inhibition will be recorded as MIC value. An satisfactory reason for this increase in antibacterial activity may be considered in the light of Overtone's concept[29] and chelation theory.[30] According to Overtone's concept of cell permeability, the lipid membrane that surrounds cell favours the passage of only lipid soluble materials, so that liposolubility is an important factor which controls bacteriostatic activity. On chelation, the polarity of the Ru(III) ion will be lowered to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the Ru(III) ion with donor groups. Further, it increases the delocalization of π-electrons over whole chelate ring and enhances lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocks the metal binding sites in bacterial enzymes. The complexes also disturb the respiratory processes of the cell and thus block the synthesis of proteins, which restricts further growth of the organism.

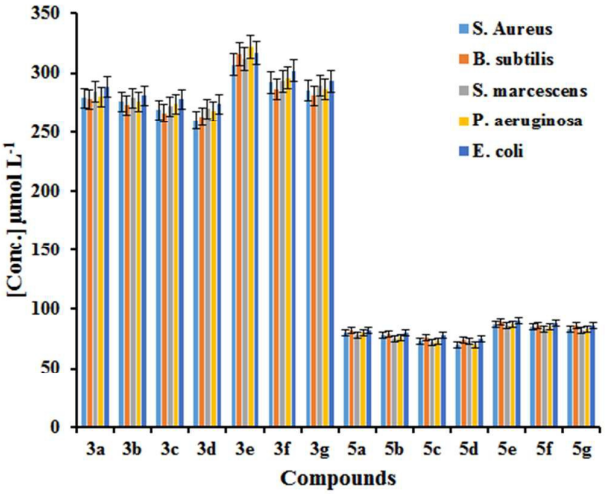


Figure 3. Effect of different concentration of free ligands and synthesized complexes on five unlike microorganisms. Error bars represent standard deviation of three replicates.

From figure 3, we observed that the antimicrobial screening of all complexes against different microorganisms better than

View Article Online
DOI: 10.1039/C6MD00251J

that of respective ligands (supplementary material 7). The results indicated that a lower concentration of complex (5d) was required to inhibit the bacterial growth and kill the bacterial strain, leading to a higher efficiency in antimicrobial activities. Complex (5d) is the most active amongst all the complexes due to presence of fluorine atom and pyridine ring substituents in quinoline ligand. Presence of more electronegative environment in complex (5d) improves its biological property. All the complexes show lower MIC value against all bacterial species than $[Ru^{III}(OFL)(PPh_3)_2Cl_2]$[27] and $[Ru^{III}(SPF)(PPh_3)_2Cl_2]$[27] but higher than $[Ru^{III}(L^{1-6})PPh_3)Cl_3]$[28] and comparable to $[Ru^{III}(L^{1-7})_3]\bullet(PF_6)_3$[11] compounds reported in published literature. All the complexes show better activity against *S. aureus*, *E. coli* and *P. aeruginosa* than those of $[Ru(phen)_3]Cl_2$, $[Ru(4,7\text{-}Me_2phen)_3]Cl_2$ and lower than $[Ru(3,4,7,8\text{-}Me_4phen)_3]Cl_2$ reported by Fangfei Li *et al.*[31] and comparable activity against *S. aureus* than those of reported by P.-L. Lam *et al.*[32]

3.4.4 DNA interactions

DNA can be regarded as a double stranded chromonic, side-chain polymer, with a core of stacked bases resembling a chromonic column, It is the π–π interactions between the bases that holds the stack together and the sugar/phosphate chains are wound round the column loosely enough to allow intercalation of a guest molecule. In order to accommodate the foreign molecule, the chain must unwind, far enough to allow two bases to move apart and make room for the intercalating molecule to enter the stack. A large number of soluble, multi-ring aromatic molecules are able to intercalate in this way. These include dyes, the fluorescent marker, ethidium bromide, many antibiotics, cancer-causing agents and anticancer drugs [33]. Since DNA is the key target for many metal-based drugs, and DNA-binding is the critical step for the study of effective metal-based drugs, which is of importance in illuminating the mechanisms involved in the site specific recognition of DNA, and designing new types of pharmaceutical molecules, the binding behaviors of the synthesized compounds toward DNA are explored both theoretically and experimentally with the aid of different procedures and techniques.

(I) By absorption spectral analysis

The binding ability of the complexes with HS DNA can be characterized by measuring their effects on electronic absorption spectra. It is one of the most useful techniques for the study of binding mode and binding strength of the compounds with DNA. Ruthenium metal complexes as well as most drugs tend to interact with DNA non-covalently through three selective modes: (I) a groove-bound fashion stabilized by a mixture of hydrophobic, electrostatic and hydrogen-bonding interactions; (II) an intercalative association with planar, heteroaromatic moieties between the DNA base pairs; and (III) electrostatic binding.[34] The intrinsic DNA binding constants *i.e.* K_b of the complexes to HS DNA were quantitatively determined by observing the change in the absorption intensity of the spectral bands by subsequent addition of HS DNA. An absorption spectrum of complexes with HS DNA was recorded for a constant concentration of complexes (20 μmol L^{-1}) with varying concentration of DNA (100 μmol L^{-1}) to obtain different DNA/complex mixing ratio is shown in figure 4.

Complexes binding to DNA through intercalation usually result in hypochromism and bathochromism called red shift [35]. When the complex intercalates with base pairs of DNA, the π* orbital of the intercalated ligand of the complex couples with π orbital of the base pairs of DNA, thus decreasing the π–π* transition energy and resulting in the bathochromism.[36] The absorption spectral changes were monitored around 270−292 nm for the investigation of DNA binding mode and strength. As the DNA concentration is increased, transition bands of complexes (5a−5g) exhibit hypochromicity [hypochromicity, H% = $[(A_{free} - A_{bound}) / A_{free}] \times 100\%$] of about 20.23−23.14%, and bathochromicity of 2−4 nm. The complex (5d) has highest percentage hypochromicity (23.14%). These spectral features may recommend a mode of binding that covers a stacking interaction between the aromatic chromophore and the DNA base pairs.

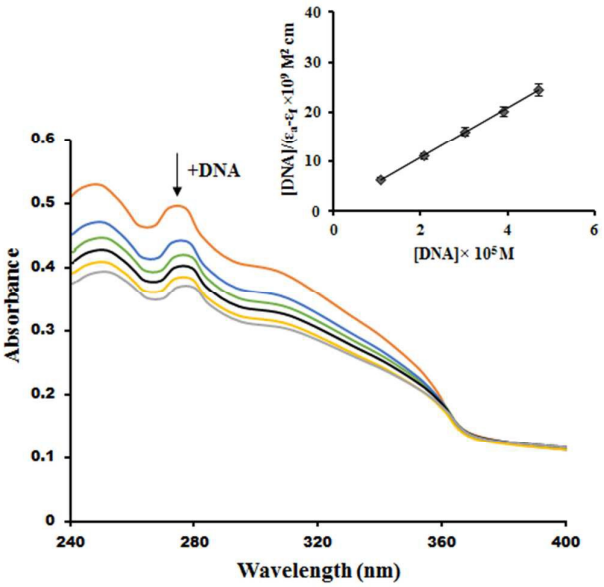


Figure 4. Absorption spectral changes on addition of HS DNA to the solution of complex (5d) after incubating it for 10 minutes at room temperature in phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH=7.2). Inset: plot of [DNA]/$(\varepsilon_a - \varepsilon_f)$ vs. [DNA]. Error bars represent standard deviation of three replicates. (Arrow shows the change in absorption with increase in concentration of DNA).

The K_b values of all compounds are found in the range 0.34×10^5 – 6.25×10^5 L mol^{-1} (supplementary material 6). It is lower than K_b value of classical intercalators ethidium bromide (7.16×10^7 L mol^{-1}), $[Ru(PEF)Cl_2(H_2O)_2]\bullet5H_2O$ (5.00×10^7 L mol^{-1})[37] and $[Ru^{III}Cl_2(PPh_3)_2L^1]$ ($1.2\pm0.14\times10^6$ L mol^{-1})[38] but higher than $[Ru^{III}(cipro)_3]\bullet4H_2O$ ($2.5\pm0.9\times10^4$ L mol^{-1}),[39] $[Ru^{III}(L^1)(H_2O)_2]Cl$ (8.72×10^4 L mol^{-1}),[40] $[Ru^{III}(L^2)(H_2O)_2]Cl$ (6.35×10^4 L mol^{-1}),[40] $[Ru^{III}Cl_2(PPh_3)_2L^2]$ ($3.9\pm0.12\times10^4$ L mol^{-1}),[38] $[Ru^{III}Cl_2(AsPh_3)_2L^2]$ ($1.6\pm0.11\times10^4$ L mol^{-1}),[38] $[RuCl(AsPh_3)L^2]$ (3.6×10^4 L mol^{-1}),[41] $[RuCl(AsPh_3)L^3]$ (3.2×10^4 L

View Article Online
DOI: 10.1039/C6MD00251J

mol^{-1})[41] and comparable with [$Ru^{III}Cl_2(AsPh_3)_2L^1$] (5.6±0.09 ×10^5 L mol^{-1}),[38] [$RuCl(AsPh_3)L^1$] (4.7×10^5 L mol^{-1}).[41] From the K_b value and red shift, it is clear that the complexes bind to the DNA by intercalation mode and complex (5d) has the highest binding ability (figure 5) than the other complexes and ligands due to an existence of fluorine atom. An existence of fluorine atoms act as chemical isosteres for the oxygen atoms in the heterocyclic base of thymidine, as a result it shows better DNA interaction activity than other. The binding mode is further confirmed by hydrodynamic volume (i.e. viscosity) measurement.

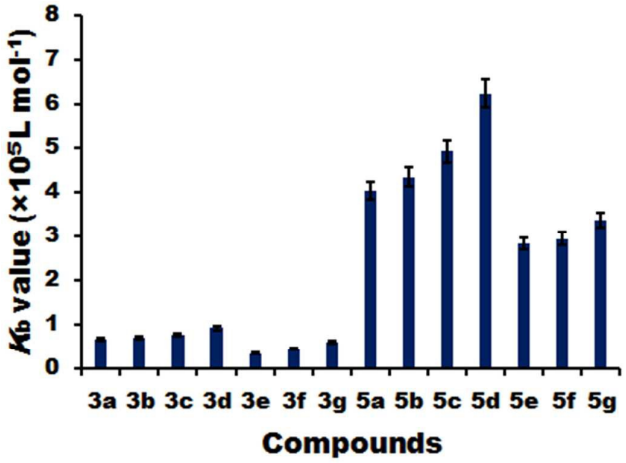


Figure 5. Plot of K_b values of synthesized compounds in L mol^{-1}. Error bars represent standard deviation of three replicates.

(II) By hydrodynamic volume or viscosity measurement

Spectroscopic study are essential, but not satisfactory to support a binding mode. The binding mode or nature of the interaction of all the compounds with HS DNA was further confirmed by viscosity measurements. A partial and non-classical ligand intercalation causes a bend in the DNA helix, reducing its effective length and thereby its viscosity. Whereas in classical intercalation, the DNA helix lengthens as base pairs are separated to accommodate the bound ligand, leading to increase in DNA viscosity. Therefore viscosity measurement is regarded as the least ambiguous and the most critical means for studying the binding mode of metal complexes with DNA in solution and provides stronger arguments for intercalative binding mode.[25] The effects of synthesized complexes on the viscosity of HS DNA are shown in figure 6.

As illustrated in this figure, on increasing the amount of the complexes the relative viscosity of HS DNA increases steadily, which confirms that the organometallic Ru(III) complexes are bound to HS DNA by intercalation. This phenomenon may be explained by the insertion of the compounds between the DNA base pairs, leading to an increase in the separation of base pairs at intercalation sites and thus an increase in overall DNA length. It is clear from the figure that all these complexes show an increase in the relative viscosity of HS DNA. The increase in the degree of viscosity of all compounds depends on their affinity to DNA, with an order as follows: 5d > 5c > 5b > 5a > 5g > 5f > 5e > quinoline ligands (supplementary material 8). This behavior is similar to that of ruthenium complexes reported by Ihtshamul Haq *et al*[42] and Caipihg. Tan *et al*.[43, 44] The viscosity results may reveal the tendency of each ligand to intercalate into DNA base pairs. The increase in DNA viscosity observed in the complexes suggests a classical intercalative mode.

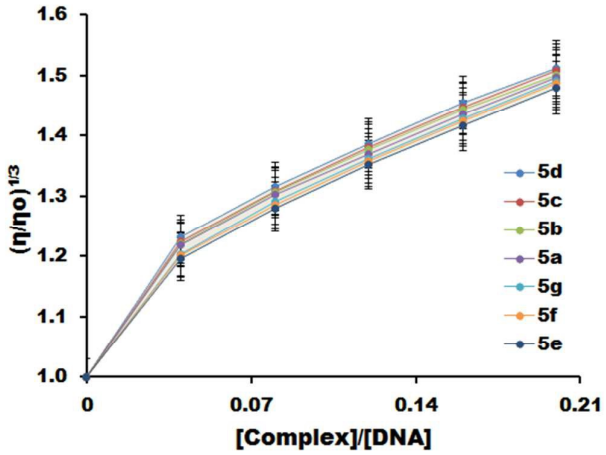


Figure 6. Effect on relative viscosity of HS DNA under the influence of increasing amounts of complexes at 27 (±0.1) °C in phosphate buffer at pH=7.2. Error bars represent standard deviation of three replicates.

(III) By molecular docking with DNA sequence d(ACCGACGTCGGT)2

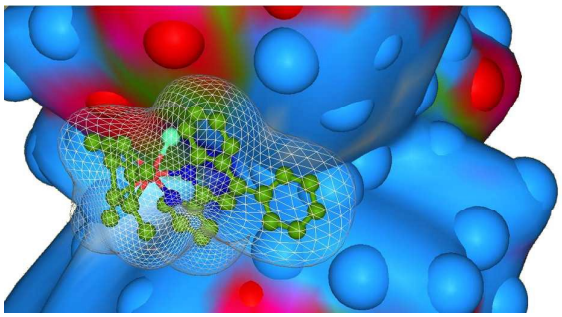


Figure 7. Molecular docking of the complex 5a (ball and stick) with the DNA duplex (VDW spheres) of sequence d(ACCGACGTCGGT)2. The complex is docked in to the DNA showing intercalation between the DNA base pairs.

Molecular docking studies of the compounds with the DNA duplex were performed to predict the chosen binding site along with the preferred orientation of compound inside the DNA helix which is shown in figure 7. To understand the preferred orientation of sterically acceptable compound with the DNA sequence at the theoretical level, molecular docking study was performed. By placing a small molecule into the binding site of the target specific area of DNA, this study has played important roles in the mechanistic study as well as understanding the metal based drug-DNA interactions for rational drug discovery and design. The study shown that the compounds under investigation interact with DNA via an

View Article Online
DOI: 10.1039/C6MD00251J

intercalation mode involving outside edge stacking interaction with oxygen atom of the phosphate backbone. From the resultant docked models, it is clear that the compounds fit well into the intercalative mode of the targeted DNA and A–T rich region stabilized by van der Waal's interaction and hydrophobic contacts.[45, 46] The resulting binding energies of docked complexes (5a–5g) are found to be −274.94, −295.61, −300.01, −327.01, −310.34, −319.77 and −325.71 kJ mol^{-1}, respectively. Also the binding energies of docked compounds (3a–3g) are found to be −232.08, −244.75, −242.87, −252.56, −229.80, −246.00 and −250.55 kJ mol^{-1}, respectively. All the complexes show comparable result to $[Ru^{III}(L^{1-7})_3]\bullet(PF_6)_3$ compounds reported in published literature.[11] Therefore, molecular docking together with absorption spectral and viscosity studies indicate that the compound interacts with the DNA through the classical intercalation mode mainly inside the DNA helix.

(IV) By agarose gel electrophoresis: photo quantization technique

There has been considerable interest in DNA nucleolytic cleavage reactions that are activated by complexes. Cleavage study on plasmid DNA induced by synthesized compounds can be monitored by agarose gel electrophoresis using pUC19 DNA. Agarose gel electrophoresis is a convenient method to assess cleavage of DNA by metal based drugs to assess the factors affecting the nucleolytic efficiency of a compound and to compare the nucleolytic property of different compounds. The DNA cleavage can occur by hydrolytic and oxidative pathways, in which hydrolytic DNA cleavage involves cleavage of phosphodiester bond to generate fragments which can be subsequently relegated; started in a modest way of converting supercoil (SC) form of DNA to the open-circular (OC) form and last in linear (L) form, is now being used for identifying the percentage of cleavage as a function of concentration of nuclease. Oxidative DNA cleavage involves either oxidation of the deoxyribose moiety by abstraction of sugar hydrogen or oxidation of nucleobases.[29]

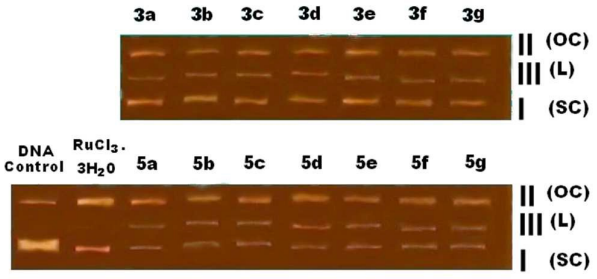


Figure 8. Photogenic view of cleavage of pUC19 DNA (300 μg/cm^3) with series of compounds using 1% agarose gel containing 0.5 μg/cm^3 EtBr. All reactions were incubated in TE buffer (pH 8) at a final volume of 15 mm^3 for 24 h at 37 °C.

The principle of this method is that molecules migrate in the gel as a function of their mass, charge and shape, with supercoiled DNA migrating faster than open circular molecules of the same mass and charge. The native DNA remains in the supercoiled (SC) form, also known as covalently coiled coil DNA, here designated as Form I. Single strand cleavage results in so called nicked or open circular (OC) form of DNA (designated as Form II), whereas the double-strand cleavage results in linear (LC) form of DNA (designated as Form III). The migration rate during agarose gel electrophoresis depends on both size (base pairs) and conformation, with smaller or condensed DNA migrating faster than larger or unfolded DNA. Form I has a tightly packed conformation and therefore migrates faster through agarose gels than linear DNA (intermediate migration) or open circle DNA (slowest migration).

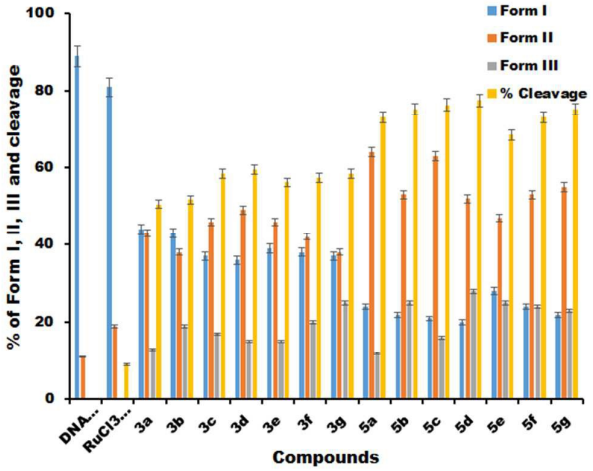


Figure 9. Plot of DNA cleavage data by agarose gel electrophoresis of different compounds using pUC19 DNA. Error bars represent standard deviation of three replicates.

Figure 8 shows the electrophoretic separation of pUC19 DNA when reacted with compounds under aerobic conditions. This clearly show that the relative binding efficacy of compounds to DNA is much higher than the binding efficacy of ruthenium salt and free quinoline ligands (figure 9 and supplementary material 10). The difference in DNA-cleavage efficiency of complexes was due to the difference in binding affinity of complexes to DNA. The similar behavior of Ru(III) complexes with plasmid DNA was shown by reported compounds of type $[Ru^{III}(PFL)(PPh_3)_2Cl_2]$,[27] $[Ru^{III}(OFL)(PPh_3)_2Cl_2]$,[27] $[Ru^{III}(SFL)(PPh_3)_2Cl_2]$,[27] mer-$[Ru^{III}Cl_3(CH_3CN)(dpq)]$[43] and mer-$[Ru^{III}Cl_3(dmso)(N–N)]$.[44]

3.4.5 *In vitro* antimalarial screening

Malaria cause vast medical, financial, and emotional problem in the world. *Plasmodium falciparum* is the parasite responsible for most malaria cases up to 80%, which often proves harmless. Malaria is one of the most prevalent infectious diseases worldwide and it represents a major global health issue for which new effective chemotherapies are urgently needed. As part of this search for novel drugs against malaria, we report encouraging results for compounds resulting from the modification of quinoline moiety by coordination to ruthenium metal centres; the new compounds

View Article Online
DOI: 10.1039/C6MD00251J

are highly active against a chloroquine resistant strain of *Plasmodium falciparum*. The results of the pharmacological screening are expressed as the drug concentration resulting in 50% inhibition (IC_{50}) of parasite growth. The mean values of IC_{50} in mg/L for compounds are given in supplementary material 6 (0.55–1.84 mg/L).

Figure 10 shows that all complexes exhibit good antimalarial activity than that of respective ligands. It is thus clear that the combination of ruthenium metal and quinoline ligand in a single molecule does produce an improvement of the activity against resistant strains of the parasite, demonstrating the validity of our concept in the search for novel antimalarial drugs capable of overcoming resistance. The IC_{50} values of all Ru(III) complexes are comparable to $[Ru^{III}(L^{1\text{-}7})_3]\bullet(PF_6)_3$,[11] $[Ru(A)_2(B)]Cl_2$ (10 mg/L),[47] [Cu(terpyridyl)Cl]Cl (0.52 µmol L^{-1}) and Fe(terpyridyl)Cl_3 (0.63 µmol L^{-1}).[48]

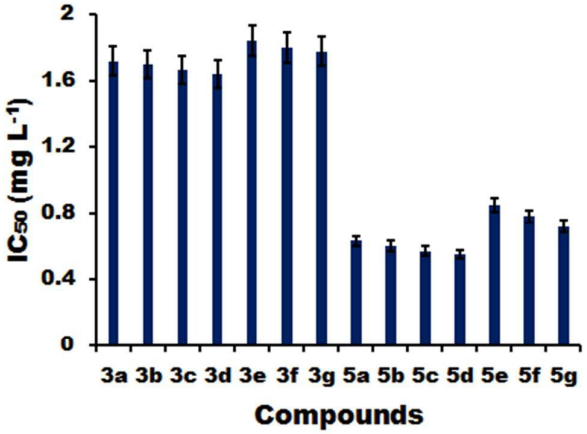


Figure 10. Plot of IC_{50} values of different compounds in mg/L using *Plasmodium falciparum* strain. Error bars represent standard deviation of three replicates.

4. Final Remarks

A series of substituted quinoline nucleus based half-sandwich organo-ruthenium complexes were synthesized and well characterized., in search of new metal based drugs looking promising as potent cytotoxic, DNA binding, DNA cleavage, antimicrobial as well as antimalarial agents. This synthetic approach were carried out with an aim to study their biological activity and allows the incorporation of potent bioactive nuclei in a single skeleton through an easy way. Likewise arene ring, pentamethylcyclopentadienyl ring derivatives occupy three coordination sites at the octahedral metal centre and provide to the metal a lipophilic protecting face. On the other hand, the remaining three coordination sites allow the introduction of ligands with hydrophilic character. By analogy with their structure to a piano stool these are known as piano stool complexes, with the metal centre being coordinated by pentamethylcyclopentadienyl ring, a chlorido ligand and a chelating quinoline ligand. The molar conductivities values of all organometallic Ru(III) complexes are indicates, one counter ion present outside the coordination sphere which accomplish all complexes are ionic in nature. All the complexes show strong *in vitro* cytotoxic, *in vitro* antimalarial as well as cellular level bioassay compared to free ligands. The antimicrobial activity of the all compounds has been tested on five dissimilar microorganisms and the data show an improved biological activity of all complexes in relation to the free ligands. Hypochromism and bathochromism (red sift) of band in absorption spectral titration and increase in relative viscosity of DNA recommend that all complexes bind with DNA via classical intercalative mode.

Ligand (3d) is the most active amongst all the ligands due to presence of fluorine atom and pyridine ring substituents in quinoline ligand. Presence of more electronegative environment in ligand (3d) improves its biological property. Therefore, complex (5d) of ligand (3d) is more potent than other complexes. Presence of fluorine atoms act as not only chemical isosteres for the oxygen atoms in the heterocyclic base of thymidine, but shows strong affinity of binding due to strong van der Waals force of interaction between fluorine and hydrogen atom compared to other substituents as a result it shows superior antimicrobial and DNA interaction activity than other compounds. Molecular docking studies of the synthesized compounds with the DNA duplex were performed to predict the chosen binding site, which suggests intercalation between compounds and DNA base pairs. The DNA cleavage study of pUC19 shows that all complexes have high cleavage ability than metal salt and ligands. Presence of more electronegative environment in complex (5d) improves its biological property. Hence, we concluded that pyridine substituted quinoline is more potent than thiophene substituted quinoline because pyridine contain nitrogen atom is more electronegative than sulphur atom. The preliminary studies encourage for carrying out further *in vivo* experiments. The results are of importance towards further designing and developing ruthenium based complexes and systematic assessment of biological activity for their potential applications as therapeutic agents.

Acknowledgements

The authors thankful to the Head, Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India, for providing the laboratory facilities, Dhanji P. Rajani, microcare laboratory, Surat for *in vitro* antimalarial activity, IIT SAIF, Bombay for EPR analysis, U.G.C., New Delhi for providing financial assistance of UGC BSR grant No. C/2013/BSR/Chemistry/59 and "BSR UGC One Time Grant", vide UGC letter No.F.19-119/2014(BSR).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

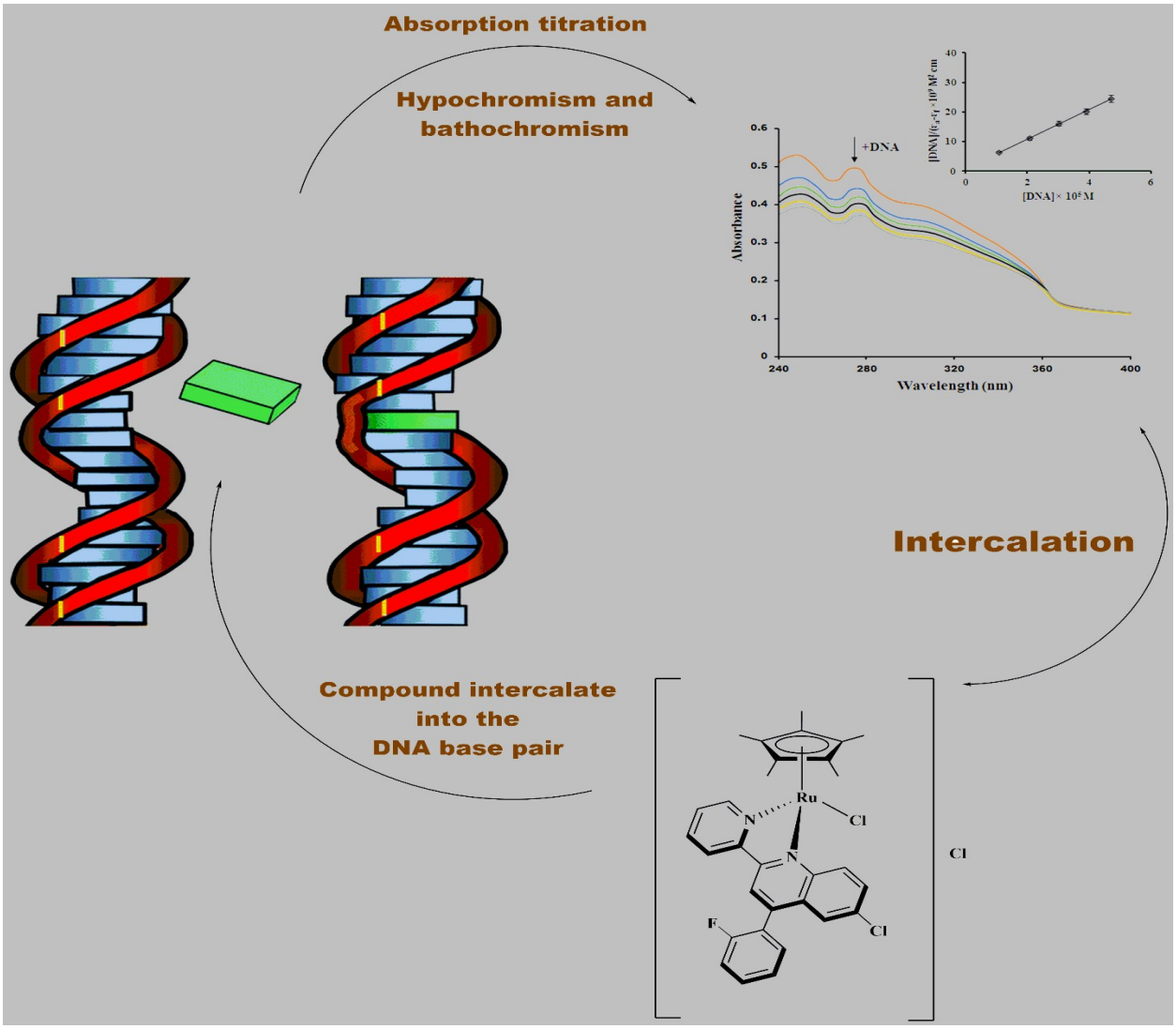
View Article Online
DOI: 10.1039/C6MD00251J

References

1 Y.-L. Chen, K.-C. Fang, J.-Y. Sheu, S.-L. Hsu and C.-C. Tzeng, *J. Med. Chem.,* 2001, **44**, 2374-2377.
2 S. Eswaran, A. V. Adhikari and R. Ajay Kumar, *Eur. J. Med. Chem.,* 2010, **45**, 957-966.
3 M. Zora and Ö. Velioğlu, *J. Organomet. Chem.,*2008, **693**, 2159-2162.
4 E. David, S. Pellet-Rostaing and M. Lemaire, *Tetrahedron*, 2007, **63**, 8999-9006.
5 B. Gabriele, R. Mancuso, G. Salerno, G. Ruffolo and P. Plastina, *J. Org. Chem.,* 2007, **72**, 6873-6877.
6 J. E. Weder, C. T. Dillon, T. W. Hambley, B. J. Kennedy, P. A. Lay, J. R. Biffin, H. L. Regtop and N. M. Davies, *Coord. Chem. Rev.,* 2002, **232**, 95-126.
7 S. H. van Rijt and P. J. Sadler, *Drug Discovery Today*, 2009, **14**, 1089-1097.
8 C. G. Hartinger and P. J. Dyson, *Chem. Soc. Rev.*, 2009, **38**, 391-401.
9 M. J. Clarke, *Coord. Chem. Rev.,* 2003, **236**, 209-233.
10 S. P. Fricker, *Dalton Trans.*, 2007, DOI: 10.1039/B705551J, 4903-4917.
11 J. V. Mehta, S. B. Gajera, P. Thakor, V. R. Thakkar and M. N. Patel, *RSC Adv.,* 2015, **5**, 85350-85362.
12 A. Bolhuis, L. Hand, J. E. Marshall, A. D. Richards, A. Rodger and J. Aldrich-Wright, *Eur. J. Pharm. Sci.*, 2011, **42**, 313-317.
13 Y.-B. Zeng, N. Yang, W.-S. Liu and N. Tang, *J. Inorg. Biochem.,* 2003, **97**, 258-264.
14 I. Meistermann, V. Moreno, M. J. Prieto, E. Moldrheim, E. Sletten, S. Khalid, P. M. Rodger, J. C. Peberdy, C. J. Isaac, A. Rodger and M. J. Hannon, *Proc. Natl. Acad. Sci.,* 2002, **99**, 5069-5074.
15 C.-Y. Zhou, J. Zhao, Y.-B. Wu, C.-X. Yin and Y. Pin, *J. Inorg. Biochem.,* 2007, **101**, 10-18.
16 F. Gao, H. Chao, F. Zhou, Y.-X. Yuan, B. Peng and L.-N. Ji, *J. Inorg. Biochem.,* 2006, **100**, 1487-1494.
17 B. A. Chabner and T. G. Roberts, *Nat Rev Cancer*, 2005, **5**, 65-72.
18 A. Chaubey and S.N.Pandeya, *Asian J Pharm Clin Res.,* 2011, **4**, 5-8.
19 R. Mishra, K. K. Jha, S. Kumar and I. Tomer, *Der Pharma Chemica*, 2011, **3**, 38-54.
20 M. M. T. Khan, D. Srinivas, R. I. Kureshy and N. H. Khan, *Polyhedron*, 1991, **10**, 2559-2565.
21 C. J. Ballhausen, *Introduction to Ligand Field Theory*, McGarw Hill, New York, 1962.
22 A. B. P. Lever, *Inorganic Electronic Spectroscopy*, Elsevier, New York, 2 edn., 1984.
23 R. K. Gupta, R. Pandey, G. Sharma, R. Prasad, B. Koch, S. Srikrishna, P.-Z. Li, Q. Xu and D. S. Pandey, *Inorg. Chem.*, 2013, **52**, 3687-3698.
24 M. Yadav, A. K. Singh and D. S. Pandey, *J. Organomet. Chem.*, 2011, **696**, 758-763.
25 J. V. Mehta, S. B. Gajera, D. D. Patel and M. N. Patel, *App. Organome. Chem.*, 2015, **29**, 357-367.
26 B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols and J. L. McLaughlin, *Planta Med*, 1982, **45**, 31-34.
27 M. Patel, H. Joshi and C. Patel, *J Chem Sci*, 2014, **126**, 739-749.
28 P. S. Karia, P. A. Vekariya, A. P. Patidar, R. R. Patel and M. N. Patel, *Monatsh. Chem.,* 2016, DOI: 10.1007/s00706-016-1708-8, 1-12.
29 J. V. Mehta, S. B. Gajera and M. N. Patel, *Spectrochim. Acta, Part A,* 2015, **136, Part C**, 1881-1892.
30 B. G. Tweedy, *Phytopathalogy*, 1964, **55**, 910.
31 F. Li, Y. Mulyana, M. Feterl, J. M. Warner, J. G. Collins and F. R. Keene, *Dalton Trans.*, 2011, **40**, 5032-5038.
32 P. L. Lam, G. L. Lu, K. M. Hon, K. W. Lee, C. L. Ho, X. Wang, J. C. O. Tang, K. H. Lam, R. S. M. Wong, S. H. L. Kok, Z. X. Bian, H. Li, K. K. H. Lee, R. Gambari, C. H. Chui and W. Y. Wong, *Dalton Trans.*, 2014, **43**, 3949-3957.
33 K. Mundy, J. C. Sleep and J. E. Lydon, *Liquid Crystals*, 1995, **19**, 107-112.
34 C. V. Kumar and E. H. Asuncion, *J. Am. Chem. Soc.,* 1993, **115**, 8547-8553.
35 J. R. J. Sorenson, *J. Med. Chem.*, 1984, **27**, 1747-1749.
36 R. Indumathy, S. Radhika, M. Kanthimathi, T. Weyhermuller and B. Unni Nair, *J. Inorg. Biochem.,* 2007, **101**, 434-443.
37 H. Muslu, A. Golcu, M. Tumer and M. Ozsoz, *J. Coord. Chem.*, 2011, **64**, 3393-3407.
38 K. Sampath, S. Sathiyaraj, G. Raja and C. Jayabalakrishnan, *J. Mol. Struct.*, 2013, **1046**, 82-91.
39 M. K. Tanimoto, K. Dias, S. Dovidauskas and S. Nikolaou, *J. Coord. Chem.*, 2012, **65**, 1504-1517.
40 A. A. Abdel Aziz and H. A. Elbadawy, *Spectrochim. Acta, Part A*, 2014, **124**, 404-415.
41 G. Prakash, R. Manikandan, P. Viswanathamurthi, K. Velmurugan and R. Nandhakumar, *J. Photochem. Photobiol. B*, 2014, **138**, 63-74.
42 I. Haq, P. Lincoln, D. Suh, B. Norden, B. Z. Chowdhry and J. B. Chaires, *J. Am. Chem. Soc.,* 1995, **117**, 4788-4796.
43 C. Tan, J. Liu, H. Li, W. Zheng, S. Shi, L. Chen and L. Ji, *Journal of Inorganic Biochemistry*, 2008, **102**, 347-358.
44 C. Tan, J. Liu, L. Chen, S. Shi and L. Ji, *J. Inorg. Biochem.,* 2008, **102**, 1644-1653.
45 E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng and T. E. Ferrin, *J. Comput. Chem.*, 2004, **25**, 1605-1612.
46 S. Tabassum, M. Zaki, M. Afzal and F. Arjmand, *Dalton Trans.*, 2013, **42**, 10029-10041.
47 S. S. Anchuri, S. Thota, R. N. Bongoni, R. Yerra, R. N. Reddy and S. Dhulipala, *J. Chin. Chem. Soc.*, 2013, **60**, 153-159.
48 J. Paraskevopoulosa, P. Smithc, H. Hopped, D. Choprae, T. Govenderb, H. Krugera and G. Maguirea, *S. Afr. J. Chem.*, 2013, **66**, 80-85.

View Article Online
DOI: 10.1039/C6MD00251J

Graphical abstract and synopsis



"Spectral changes accompanying addition of HS DNA to solution of quinoline nucleus based piano stool coordination compounds indicate intercalative mode of binding of the compound between DNA base pairs."