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Synthesis, Antibacterial, Molecular Docking, DNA Binding and Photonuclease Activity of Quinoline Isoxazoles

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ABSTRACT

Isoxazole nuclease is a constituent of many bioactive heterocyclic compounds that are of wide interest because of their diverse biological and clinical applications. The antibacterial, In-silico molecular docking, DNA binding, thermal denaturation, viscosity measurements and photonuclease activity of newly synthesized quinoline isoxazole containing SH/SeH were described. Antibacterial potential of these compounds screened against a wide range of Gram-positive and Gram-negative bacteria showed significant zone of inhibition and MIC with standard drug ciproflaxin were studied. In molecular docking second orientation exibit binding, docking energy with inhibition constant. The absorbance plot showed active interaction of parent isoxazole derivative with CT DNA as efficient DNA intercalator (K_b = 3.8×10^5 M^{-1}). The viscosity measurements and thermal denaturation studies afford the positive results in intercalation with DNA. The light induced DNA damage is pragmatic in the absence of various "inhibitors" showed on photo cleavage studies at 360 nm.

Keywords: *In-silico* Molecular docking, intercalation, Photocleavage.

INTRODUCTION

Heterocyclic compounds, containing one or two heteroatoms, fused to quinoline ring in a linear fashion are found in natural products, as well as in the derivatives of isoxazole have fascinated extensively in synthetic and medicinal interest[1-4]. The construction of the isoxazoles rings by 1,3-dipolar cycloaddition of a nitrile oxide to an alkene or alkyne has proven to be extremely productive in synthetic chemistry[5,6]. The propagyl glycines are versatile synthetic building blocks which had recently initiate application as multifunctional substrates for "click chemistry" [7].

The isoxazoles are known to exhibit anti-malarial, anti-inflammatory, anti-asthmatic, anti-bacterial, anti-hypertensive and platelet derived growth factor receptor tyrosine kinase (PDGF-RTK) inhibiting agents[8,9],antiallergenic, anticancer[10], antifungal[11], hypocholesterolemic, hypolemic, antibacterial, and antiviral properties[12] In addition, various fused system of quinolines were studied for their intercalative DNA binding properties.

A literature survey reveals the antitumor activity is due to the intercalation between the base pairs of DNA and interferences with the normal functioning of enzyme topoisomerase II, which is involved in the breaking and releasing of DNA strands[13]. The biological and pharmaceutical activities of different S/Se compounds are of special interest since they are active sites of a large number of sulphur and selenium dependent enzymes and help in prevention of cancer[14-16].

Results of these various binding studies reveals that intercalation with the DNA which has been useful in designing new and promising anticancer agent for clinical use. The interaction of DNA with small molecules is basic work in the design of new types of pharmaceutical molecules. Studies on the interaction model and the mechanism of DNA cleavage, and exploring the application in antineoplastic medication, molecular biology and bioengineering were hotspots in these recent years.

In addition, some isoxazoles and their derivatives often have diverse biological and pharmaceutical activities. However, up to now the biological activity and interactions of newly synthesized isoxazoles with DNA have not been reported. This aroused our interest in the synthesis of Quinoline isoxazole and its derevatives in view of evaluating their DNA-binding and cleavage properties.

MATERIALS AND METHODS

The melting points are determined by open capillary methods and are uncorrected. The UV-Visible spectra are recorded on a Shimadzu model impact 1650 UV-Visible double beam spectrometer. The FT-IR spectra are recorded on a Shimadzu model impact 8400S FT-IR spectrometer (KBr pellets, 3cm^{-1} resolution), ^{1}H NMR spectra on a Bruker 400 MHz and mass spectra are recorded on ESMS Bruker Daltonics 800 MHz spectrometer. Elemental analyses are done on Vario EL.CHNOS elemental analyser. Viscosity measurements are studied by semi micro dilution capillary viscometer (Viscomatic Fica MgW) with a thermostated bath D40S. Unless or otherwise mentioned, chemicals of AR grade are purchased from himedia pvt. Ltd. amino acids used are L-configuration. All solvents are freshly distilled. CTDNA is purchased from Bangalore Gene, Bangalore, India. Tris–HCl buffer (5mMTris–HCl, 50M NaCl, pH = 7.2, Tris=Tris (hydroxymethyl) amino methane) solution is prepared using deionized double-distilled water. TLC analysis is carried out using the precoated silica gel GF_{454} plates.

General Experimental Procedure

The derivatives 4(a-g) of quinoline carbaldehyde (10 mmol) is dissolved in a solution of hydroxyl amine hydrochloride (11mmol) in 40 ml of 3:1 *t*-BuOH: H_2O.To this NaOH (11 mmol) is added and stirred for 30 min at room temperature .Then TLC were analyzed for the formation of oxime. This is continued by addition of chloroamine-T trihydrate (11 mmol) in a interval of 3-4 min followed by the $CuSO_4$ (0.4mmol) with copper turnings (40mg). 2-(9H-Fluoren-9-yl-methoxy-carbonyl amino)-propionic acid prop-2-ynyl ester (10 mmol) is added, at pH-6 is adjusted by addition of 1M KOH and stirred for another 3-4 h. The completion of reaction is checked by TLC. After the completion of the reaction mixture is poured in to ice and then NH_4OH added to remove the excess of copper salts[17]. The product were isolated by aqueous workup or by filtration, dried and recrystallized in ethyl acetate. (Pg-protecting group)

2-(9H-Fluoren-9-yl-methoxycarbonylamino)-propionicacid-3-(2-chloro-quinolin-3yl)-isoxazol-5yl-methyl ester 4a: Pale green solid, yield: 84%, m.p. 128–130^{o}C, IR (ν) (KBr,cm^{-1}) 3240(-NH-), 2850(C-H str), 1650(C=O), 1610(C=N), 1570, 1550, 1460 (Ar-C=C str), 1034.8(C-O-C), 643.5(C-Cl). ^{1}H NMR (DMSO-d_6, ppm) δ: 1.48 (d, 3H, *J* = 2.8 Hz, CH_3), 4.46(t, 1H, -CH), 4.6(m, 1H, –CHpg), 4.78(d, 2H, *J*=3.2Hz,-CH_2), 5.4(s, 2H, –CH_2), 6.3(s, 1H, -CH-), 7.28-7.84 (m, 11H, Ar-H), 8.05 (d, 1H, *J* = 8.7 Hz, Ar-H), 8.5(s, 1H, Ar-H), 8.8(bs, 1H, NH) ppm. ES-MS *m/z*: ^{35}Cl: 554.90, ^{37}Cl: 556.90 $[M+H]^+$. Anal. calcd for $C_{31}H_{24}ClN_3O_5$: C, 67.24; H, 4.35; N, 7.56. Found: C, 67.21; H, 4.37; N, 7.58.

2-(9H-Fluoren-9-yl-methoxycarbonylamino)-propionicacid-3-(2-hydroxy-quinolin-3yl)-isoxazol-5yl-methyl ester 4b:Yellowish green solid, yield: 81%, m.p. 142-144^{o}C, IR (ν) (KBr,cm^{-1}) 3244(-NH-), 3385(Ar-OH), 2848(C-H str), 1648(C=O), 1608(C=N), 1572, 1548, 1462 (Ar-C=C str), 1032.8 (C-O-C). ^{1}H NMR (DMSO-d_6, ppm) δ:1.46(d, 3H, *J* = 2.9 Hz, CH_3), 4.48(t, 1H, -CH), 4.65(m, 1H, –CHpg), 4.71(d, 2H, *J*=3.2Hz,-CH_2-), 5.0(s, 1H, -OH), 5.34(s, 2H, –CH_2), 6.3(s, 1H, -CH-), 7.26-7.82 (m, 11H, Ar-H), 7.91(d, 1H, *J* = 8.8 Hz, Ar-H), 8.17(s, 1H, Ar-H), 8.6(s, broad, 1H, NH) ppm. ES-MS *m/z*: 536.5 $[M+H]^+$. Anal. calcd for $C_{31}H_{25}N_3O_6$**:** C, 69.54; H, 4.69; N,7.81**.** Found: C, 69.52; H, 4.71; N, 7.85.

***2-(9H-Fluoren-9-yl-methoxycarbonylamino)-propionicacid-3-(2-mercapto-quinolin-3yl -isoxazol-5-yl methyl ester 4c*:** Yellowish brown solid, yield: 72%, m.p. 102-104^{o}C, IR (ν) (KBr,cm^{-1}) 3241(-NH-), 3337.9(C-SH), 2852(C-H str), 1655(C=O), 1610(C=N), 1578, 1552, 1458 (Ar-C=C str), 1032.8 (C-O-C). ^{1}H NMR (DMSO-d$_6$, ppm) δ: 1.44(d, 3H, *J* = 2.9 Hz, -CH$_3$), 4.45(t, 1H, -CH-), 4.65(m, 1H, –CH-pg), 4.74(d, 2H, *J*=2.9Hz,CH$_2$), 5.32 (s, 2H, –CH$_2$), 6.3(s, 1H,-CH-), 7.26-7.82 (m, 11H, Ar-H), 7.98(d, 1H, *J* = 8.2 Hz,Ar-H), 8.05(s, 1H, Ar-H), 8.6(s, broad, 1H, NH), 13.0(s, 1H, -SH) ppm. ES-MS *m/z*: 552.5 [M+H]$^{+}$. Anal. calcd for $C_{31}H_{25}N_3O_5S$: C, 67.52; H,4.55; N, 7.64. Found: C, 67.50; H, 4.57; N, 7.62.

2-(9H-Fluoren-9-yl-methoxycarbonylamino)-propionicacid-3-(2-seleno-quinolin-3yl)-isoxazol-5-yl-methyl ester 4d: White solid, yield: 78%, m.p. 122-124^{o}C, IR (ν) (KBr,cm^{-1}) 3243(-NH-), 2605(C-SeH), 2850(C-H str), 1652(C=O), 1612(C=N), 1578, 1552, 1458 (Ar-C=C str), 1034.8 (C-O-C). ^{1}H NMR (DMSO-d$_6$, ppm) δ: 1.42(d, 3H, *J* = 3.2 Hz, CH$_3$), 4.45(t, 1H, -CH-), 4.65(m, 1H, –CH-pg), 4.71(d, 2H, *J*=3.1Hz, -CH$_2$-), 5.36(s, 2H, –CH$_2$), 6.34(s, 1H,-CH-), 7.26-7.82 (m, 11H, Ar-H), 8.1(d, 1H, *J* = 7.9 Hz,Ar-H), 8.2(s, 1H, Ar-H), 8.6(s, broad, 1H, NH), 5.8 (tautomeric form of -NH, SeH) ppm. ESMS *m/z*: 599.5 [M+H]$^{+}$. Anal. calcd for $C_{31}H_{25}ClN_3O_5Se$; C, 62.21; H, 4.21; N, 7.02. Found: C, 62.21; H, 4.23; N, 7.06.

2-(9H-Fluoren-9-yl-methoxycarbonylamino)-propionicacid-3-(2-chloro-4-methyl-quinolin-3-yl)-isoxazol-5yl methyl ester 4e: Pale green solid, yield: 84%, m.p. 156-158^{o}C, IR (ν) (KBr,cm^{-1}) 3242(-NH-), 2854(C-H str), 1648(C=O), 1608(C=N), 1580, 1548, 1462(Ar-C=C str), 1036.8(C-O-C), 640.5 (C-Cl). ^{1}H NMR (DMSO-d$_6$, ppm) δ:1.46(d, 3H, *J* = 4.2 Hz, CH$_3$), 2.42(s, 3H, -CH$_3$), 4.48(t, 1H, -CH-), 4.60(m, 1H, –CH-pg), 4.78(d, 2H, *J*=3.2, -CH$_2$), 5.38 (s, 2H, –CH$_2$), 6.32 (s, 1H,-CH-) 7.28-7.84 (m, 10H, Ar-H), 8.1(d, 1H, *J* = 8.2 Hz, -CH$_2$), 8.2(s, 1H, Ar-H), 8.6 (s, broad, 1H, NH) ppm. ESMS *m/z*: ^{35}Cl: 569.1. ^{37}Cl:571.1 [M+H]$^{+}$. Anal. calcd for $C_{32}H_{26}ClN_3O_5$: C, 67.66; H, 4.61; N, 7.40. Found: C,67.65; H, 4.62; N, 7.39.

***2-tert-butoxycarbonylamino-4-methyl-pentanoic-acid-3-(2-chloro-quinolin-3yl)isoxazol-5yl-methyl ester 4f*:** Brown solid, yield: 76%, m.p. 98-100^{o}C, IR (ν) (KBr,cm^{-1}) 3241(-NH-), 2848(C-H str), 1652(C=O),1606(C=N), 1576, 1546, 1458(Ar-C=C str), 1038.8(C-O-C), 635.5 (C-Cl)**.** ^{1}H NMR (DMSO-d$_6$, ppm) δ: 0.96(q,1H,CH), 1.33(dd, 6H, *J* = 3.4, 5.8 Hz, 2CH$_3$), 1.40(s, 9H,(-3CH$_3$), 4.48(q, 1H,-CH-), 5.32(q,2H), 7.43(t, 1H, Ar-H), 7.61(t, 1H,Ar-H), 7.68 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.51(s, 1H, Ar-H), 8.8(bs, 1H, NH)ppm. ESMS *m/z*: 474.9[M+H]$^{+}$. Anal. calcd for $C_{24}H_{28}ClN_3O_5$: C, 60.82; H,5.90;N, 8.89. Found: C,60.84; H, 5.95; N,8.86.

2-tert-butoxycarbonylamino-4-methyl-pentanoic acid-3-(2-hydroxy-quinolin-3yl)-isoxazol-5yl me -thyl ester 4g: Yellow solid, yield: 72%, m.p. 82-84^{o}C, IR (ν) (KBr,cm^{-1}) 3240(-NH-), 3370(Ar-OH), 2846(C-H str), 1648 (C=O), 1610 (C=N), 1576, 1546, 1458 (Ar-C=C str), 1036.8 (C-O-C). ^{1}H NMR (DMSO-d$_6$, ppm) δ: 1.01(dd, 6H, *J*=3.8, 6.0 Hz, 2CH$_3$), 1.4(s, 9H,3CH$_3$), 1.86(q, 2H,-CH$_2$) 4.42(t, 1H-CH-), 5.1(bs, 1H, -OH), 5.34(s, 2H, -CH$_2$-), 7.32(t, 1H,-CH-), 7.59(t, 1H, Ar-H),7.64(d, 1H, *J* = 9.2 Hz, Ar-H), 7.92(d, 1H, *J* = 9.8 Hz, Ar-H),8.17(s,1H, Ar-H), 8.6(s, broad, 1H, NH) ppm. ESMS *m/z*: 456.5 [M+H]$^{+}$. Anal. calcd for $C_{24}H_{29}N_3O_6$: C, 63.24; H, 6.44; N, 9.20. Found: C, 63.26; H, 6.42; N, 9.22.

Biological evalution

Bacterial strains

Of the six clinical strains three of the bacterial pathogens belong to Gram-positive S. *aureus* – ATCC-29737, and S. *pyogenes* – NCIM-2608 and Gram-negative bacteria such as P.*aeruginosa* – ATCC-20852 and B. *subtilis* – NCIM-2010 are collected from National Chemical Laboratory (NCL), Pune, India. All the bacterial microorganisms are maintained at 30^{o}C in Brain Heart Infusion (BHI) containing 17% (v/v) glycerol. Before testing, the suspensions are transferred to LB broth and cultured overnight at 37^{o}C. Inocula are prepared by adjusting the turbidity of the medium to match the 0.5 McFarland standards. Dilutions of this suspension in 0.1% peptone (w/v) solution in sterile water is inoculated on LB agar, to check the viability of the preparations[18].

Antibacterial Assay

The agar well diffusion method is used for the assessment of antibacterial activity of the test samples[19]. Medium (peptone 10 g/l, yeast extract 5 g/l, sodium chloride 10 g/l, agar–agar 15 g/l, pH 7.2) was poured into sterilized Petri dishes (90 mm diameter). LB broth containing 100 ml of 24-h incubated cultures of the respective clinical isolates and the ATCC and MTCC strains was spread separately on the agar medium. Wells are created using a sterilized cork borer under aseptic conditions. Cell count was taken using haemo-cytometer after loading 10 ml of the cell

suspension in PBS and no. of cells/mL is calculated. The final concentration of each strain was 106 cells /mL. Cultures are grown for 3 days at 37 ^{o}C and wells are made using cork borer and 100 ml (10 mg/mL) of test compounds is loaded to each wells. The reference antibacterial agent ciprofloxacin is (10 mg/mL) loaded in the corresponding wells. Plates were then incubated at 37 ^{o}C for 48 h. At the end of the incubation period, inhibition zones formed on the medium are evaluated in millimeters.

Minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations (MICs) of the newly synthesized compounds **4(a-g)** are determined by micro dilution techniques in LB broth, according to Clinical and Laboratory Standards Institute (CLSI), USA guidelines. The bacterial inoculates are prepared in the same medium with density adjusted to a 0.5 McFarland turbidity standard colony forming units and diluted 1:10 for the broth micro dilution procedure. The microtiter plates are incubated at 37 ^{o}C and MIC is determined after 24 h of incubation. The highest activity of the standard drug compared with those of our synthesized compounds **4a** and **4b** indicated potent antibacterial activity (Table 3).

***In-silico* molecular docking**

Automated docking were used to determine the orientation of synthesized compound bound with DNA. A genetic algorithm method implemented in the program AutoDock 3.0 were employed[20]. The synthesized molecules were designed and the structures were analyzed by using ChemDraw Ultra 6.0. 3D coordinates were prepared using PRODRG server[21]. The DNA structure files 3GJH were taken from PDB (*www.rcsb.org/pdb*) were edited by removing the heteroatom[22]. For docking calculations, Gasteiger Marsili partial charges were assigned to the ligands and nonpolar hydrogen atoms were merged[23].

All torsions were allowed to rotate during docking. The grid maps were centered at the major groove and were adjusted such that it accommodated the major groove of the DNA duplex at the torsion degree of freedom 0.5 units. The genetic algorithm were applied for minimization, using default parameters. The number of docking runs were 50, the population in the genetic algorithm were 250, the number of energy evaluations were 100,000, and the maximum number of interactions 10,000.

DNA interaction experiment

Spectral Measurements UV–visible absorption spectrum were recorded in a SHIMADZU, UV-1650 recording double beam spectrophotometer using quartz cuvettes of 10 mm light-path. The parameter of interaction between quinoline linked isoxazole **4a** with CT-DNA is determined spectrophotometrically. Aliquots of a concentrated DNA solution (0.18–1.125 mM) are added to a cuvette filled with **4a** solution (12–25 mM) and thoroughly mixed. Extreme care is taken to ensure that an optical reference solution were prepared in an identical manner. The binding data were expressed in the form of a Scatchard plot[24].

Absorption: $(DNA)/(\varepsilon_a - \varepsilon_f) = (DNA)/(\varepsilon_b - \varepsilon_f) + 1/(\varepsilon_a - \varepsilon_f)$ ---------- **(1)**

Where ε_a, ε_f and ε_b are the apparent, free, and bound extinction coefficients at 500 nm for compound 4a respectively. A plot of $[DNA]/(\varepsilon_b - \varepsilon_f)$ versus [DNA] gave a slope of $1/(\varepsilon_b - \varepsilon_f)$ and a γ intercept equal to $1/K_b(\varepsilon_b - \varepsilon_f)$, where K_b is the ratio of the slope to the γ intercept.

The variables r (moles of ligand bound/mole of nucleotides) and C (the molar concentration of free drug) is calculated from the absorption measurements according to the method of Peacocke and Skerrett[25]. The intrinsic binding constant Kb and the maximum number of available binding sites/nucleotide (n) are deduced from Scatchard plot.

Viscosity measurements

The DNA-binding mode of the parent compound is further investigated by viscosity measurements. Hydrodynamic measurements (e.g. viscosity, sedimentation), which are sensitive to the increase in length of DNA, and regarded as the least ambiguous and the most critical tests of binding mode in solution in the absence of crystallographic structure data. Viscosity measurements are carried out by using a semi micro dilution capillary viscometer (Viscomatic FICA MgW) with a thermostated bath D40S at 20 °C. For the viscosity experiments, samples of calf thymus DNA are sonicated[26], the fragments having an estimated molecular weight of approximately[27].

Thermal denaturation experiments

The DNA melting studies are done by controlling the temperature of the sample cell with a Shimadzu (SHIMADZU, UV-1650 PC) circulating water bath while monitoring the absorbance at 260 nm. The temperature of the solution is continuously monitored with a thermo-couple attached to the sample holder.

DNA Photocleavage experiments

The extent of cleavage of super coiled (SC) pUC19 DNA (0.5 ml, 0.5 mg) to its nicked circular (NC) form is determined by agarose gel electrophoresis in Tris-HCl buffer (50 mM, pH 7.2) containing NaCl (50 mM). The reaction volumes are held in caps of polyethylene micro centrifuge tubes, which are placed directly on the surface of a trans-illuminator (8000 mW/cm) at 360 nm. The samples are irradiated for 5 min at room temperature. After irradiation the cleavage reactions, the 40 mM compound **4(a-c)** in 18 ml buffer are photo-irradiated using monochromatic UV or visible light for 1 h followed by addition to the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol (3 ml) and finally loaded on 0.8% agarose gel containing 1.0 mg/ml ethidium bromide[28].

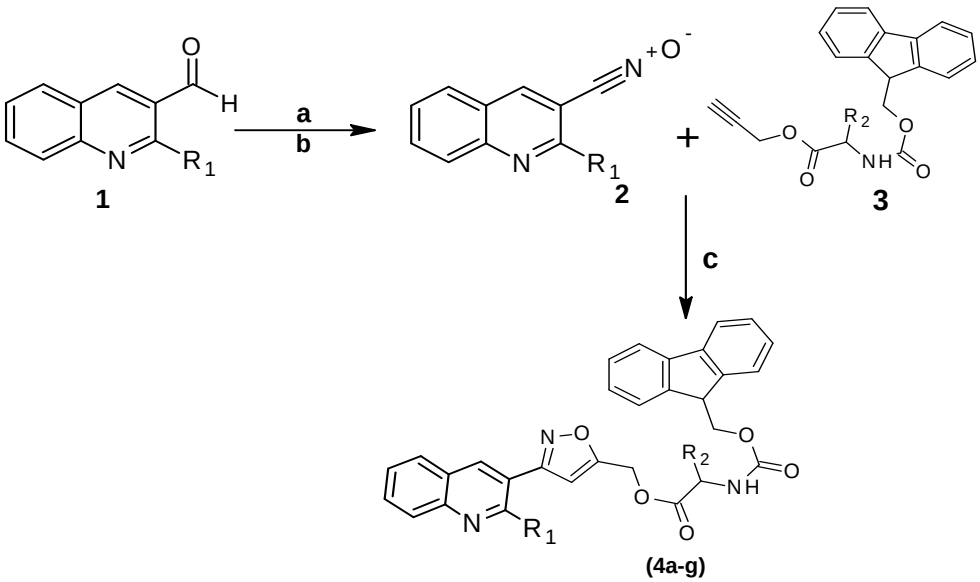
Electrophoresis is carried out at 50 V for 3 h in Tris-borate EDTA (TBE) buffer. Bands are visualized by UV light and photographed to determine the extent of DNA cleavage from the intensities of the bands using UVITEC Gel Documentation System. Because of corrections are made for the trace of NC DNA present in the SC DNA sample and for the low affinity of EB binding to SC DNA in comparison to the NC form, the wavelength used for the photo-induced DNA cleavage experiments and photographed under UV light is 360 nm[29].

RESULTS AND DISCUSSION

Chemistry

The present procedure deals with the copper(I), here this active catalyst is generated *in-situ* from the Cu(II) salts (0.25–2.0 mol %). In addition to the catalyst, a slight excess of chloroamine-T prevents formation of the oxidative coupling products that are often observed. It empowers a rapid synthesis of a isoxazole library of compounds. The Cu(I)-catalyzed, stepwise variant [30] of Huisgen's classic 1,3-dipolar cycloaddition process seems to be the best example of click chemistry reliability[31].

Scheme

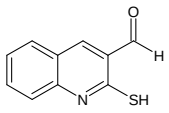
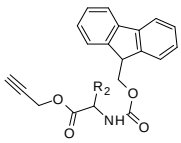
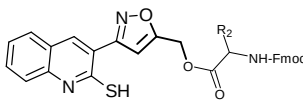
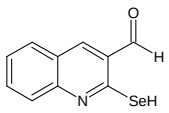
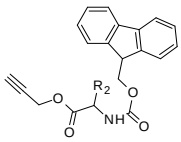
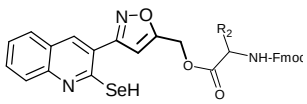
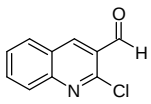
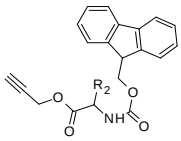
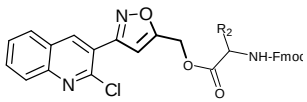
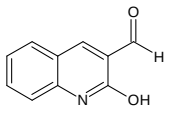
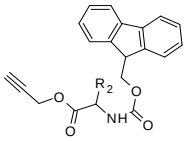
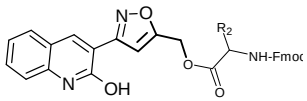
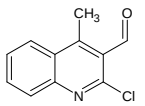
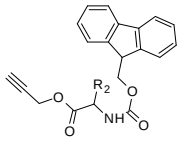
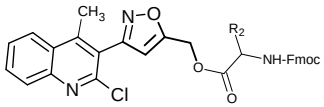
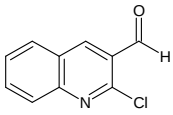
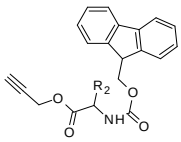
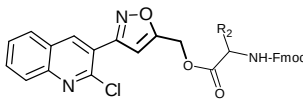
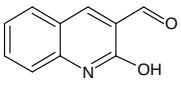
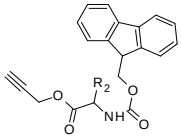
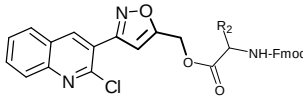


Scheme

a) $NH_2OH.HCl$, KOH. b)TsN(Cl)Na.$3H_2O$, Stirring in ambient temperature c) Cu/ $CuSO_4$ catalyst. Where R_1= SH/SeH/OH/Cl, R_2=CH_3, CH_2-CH-$(CH_3)_2$.

Our protocol demonstrated the heterocyclic aldehyde **4(a-g)** were first converted into corresponding aldoxime through reaction with hydroxylamine. The aldoxime were transformed to analogous nitrile oxide **2** using chloroamine-T trihydrate which acts as both a halogenating agent and a base[32]. Thus, nitrile oxide reacted only with the terminal Fmoc-amino acid prop-2-ynyl ester. The isolation and conduct of potentially harmful and unstable hydroximoyl chlorides is avoided. This procedure affords good yields of cyclization products with short reaction times and simple purification. The different aldehyde **4(a-g)** are from the literature[33-35].

Table 1:-Shows different derivatives using aldehyde, protecting group and yield, below

Compound.	Aldehyde (used) =R	N^{α}-Fmoc - amino acid	Product	Yield (%)
4a				84
4b				81
4c				72
4d				78
4e				83
4f				85
4g				74

The newly synthesized isoxazoles **4a-g ,** showed the IR bands around 2847-2850 cm^{-1} (C-H str) , 1610-1608 (C=N), 1648-1652(C=O), 3388-3386 (Ar-OH) and the -NH at 3243-3240 cm^{-1}, and whereas in ^{1}H-NMR showed the doublet peak at 1.48, the presence of three protons of amino acid -CH_3, at 4.46 triplet signal for the –CH group of the compound 2-(9H-Fluoren-9-methoxycarbonylamino)-propionic acid ester, 5.4 signal showed the singlet peak of propagyl –CH_2 ,the CH group of isoxazole moeity showed singlet signal at 6.34, 7.28-7.84 signals showed multiplet of aromatic protons, at 8.8 signals showed broad singlet peak which corresponds to –NH peak.

In compound [2-(9H-Fluoren-9-yl-methoxy –carbonyl amino)-propionicacid-3-(2-mercapto-quinolin-3-yl)-isoxazol-5-ylmethyl ester] **4a** showed a broad signal at 11.5 corresponds to –SH and IR at 2593 (S–H). The compound [2-

(9H-Fluoren-9-yl-methoxy carbonyl-amino)-propionicacid-3-(2-seleno-quinolin-3-yl)-isoxazol-5-ylmethylester] **4b** showed IR at 2605 (Se–H), and signal at 5.8 in the tautomeric form of **N** of quinoline with -SeH. [2-(9H-Fluoren-9-yl-methoxycarbonylamino)-propionicacid-3-(2-hydroxy-quinolin-3-yl)-isoxazol-5-ylmethylester] **4d** the –OH peak showed signal as broad singlet at 5.0 and IR absorption bands at 3385 of aromatic –OH. The compound [2-(9H-Fluoren-9-yl-methoxy-carbonyl-amino)-propionicacid-3-(2-chloro-4-methyl-quinolin-3-yl)-isoxazol-5-ylmethyl-ester] **4e** showed singlet signal at 2.42 of $-CH_3$ protons of 2-chloro-4-methyl quinoline. In the compound (**4f/4g)** showed signal at 1.33 is 6 protons of amino group.

The compound **4g** showed the broad signals of –OH proton at 5.1.The rest of the aromatic protons appeared in the usual region. Molecular ion $[M+H]^+$ peak of compound **4(a-g)** were observed at different intensities in positive ionization mode and confirmed the molecular weights of compounds with molecular ion peaks at 4a**;** 552.5, 4b**;** 599.5, **4c;** ^{35}Cl: 554.90, ^{37}Cl: 556.90, **4d;** 536.5, **4e;** ^{35}Cl: 569.1. ^{37}Cl:571.1, **4f;** 474.9, **4g;** 456.5. *m/z* values respectively. Compound which has halogen atoms in their molecule showed characteristic peaks in their mass spectrum analogous to isotopic distribution (^{35}Cl and ^{37}Cl isotopes).

Antibacterial activity

The minimum inhibitory concentrations (MICs) of the newly synthesized compound from **4a-g** with standard ciprofloxacin against all bacterial strains are as shown in Table 3. The significant MIC value is obtained for Staphylococcus *aureus*, 7.6 mg/mL for compound 4a and 8.9 mg/mL for compound 4b. Comparison of results of the antibacterial activity between newly synthesized compounds from **4a-g**, the **4a**, **4b** containing **-SH** and **-SeH** with ciprofloxacin showed a synchronizing effect on strains of pathogenic bacteria. The zones of inhibition of the bacterial colony are depicted in Table 2. Compound 4a demonstrated antibacterial activity against all the strains of bacteria. But it is significant on Gram-positive bacteria *Staphylococcus aureus* (18.20 mm) and *Streptococcus pyogenes* (17.90 mm). Among the newly synthesized compounds, compound **4a** containing sulphur component proved to be a more potent bactericidal agent against S. *aeruginosa* (17.30 mm) and Bacillus *subtilis* – NCIM- 2010 (16.40 mm). Compound **4b** containing seleno showed a meager antimicrobial property against all the pathogenic strains of bacteria.

Its bio-controlling potency is per with that of the standard antibiotic ciprofloxacin. Generally, the Gram-positive bacteria are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier[36]. Whereas the Gram-negative bacteria possess an outer phospholipidic membrane carrying the structural lipo-polysaccharide components. This makes the cell wall impermeable to drug constituents[37].

The minimum inhibitory activity is observed in Gram-positive bacteria *Staphylococcus aureus.* In the case of Gram-negative *Pseudomonas aeruginosa* the zone of inhibitory activity is significant because of a multilayered phospholipidic membrane carrying the structural lipo-polysaccharide components[38]. The compound **4a** showed maximum inhibition zones compared to that of compound 4b.Thus, compound **4a** is effective in controlling the growth of pathogenic strains to a considerable extent indicated that the compound contain **–SH** and **-SeH** group it is solely responsible for good antibacterial activity.

Table 2: Antibacterial activity of the synthesized compounds against pathogenic bacterial strains

Diameter of zone of inhibition (mm) Clinical strains				
Compounds	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus subtillis*
4a	17.90 ± 0.10	15.10 ± 0.10	17.30 ± 0.10	16.40±0.10
4b	18.20 ± 0.10	15.90 ± 0.10	14.60 ± 0.10	14.90±0.10
4c	16.60± 0.10	14.90± 0.10	15.60± 0.10	15.60±0.10
4d	16.80± 0.10	14.80± 0.10	15.10± 0.10	15.10±0.10
4e	15.90± 0.10	14.20± 0.10	14.90± 0.10	14.70±0.10
4f	16.10± 0.10	14.50± 0.10	14.50± 0.10	13.90±0.10
4g	16.40± 0.10	13.90± 0.10	14.70± 0.10	14.10±0.10
Ciproflaxin	22.80 ± 0.10	23.70 ± 0.10	22.10 ± 0.10	21.40±0.10

Table-3: MIC (mg/mL) values of synthesized compounds 4a and 4b

Bacterial strains Minimal inhibitory concentrations (MIC)-(mg/mL)				
Compounds	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus subtilis*
4a	10.9	7.6	13.2	11.5
4b	11.9	8.9	13.1	11.9
4c	12.1	9.1	13.6	12.1
4d	12.3	9.2	13.9	12.6
4e	12.2	9.4	13.4	12.5
4f	13.0	10.2	12.1	12.9
4g	12.9	9.8	12.9	11.5
Ciprofloxacin	6.45	6.65	6.15	7.25

***In-silico* molecular docking**

Careful examination of B-DNA structures reveals the reason for the difference in the positioning in the two helical forms. The predicted binding of ligands to the DNA bases on the basis of the clustering of Cartesian coordinates is thus more directional than that expected from the ellipsoids derived from Fourier averaging. Calculation of the energy of a drug-DNA system entails the enumeration of a set of critical atoms on the drug that may interact with the ligand-binding sites around the DNA bases. Each of the potential hydrogen bond donor or acceptor atoms on the drug is assigned a DNA-binding ellipsoid with complementary acceptor or donor properties and values had shown in table 4.

Table-4: Shows No. of hydrogen atoms bonds in the docking studies, Binding, docking energy and Inhibition constant of DNA docking.

Sl.No.	Compound name	No.of Hydrogen bonding	Binding energy	Docking energy	Inhibition constant
1	4a	3	-5.47	-8.88	$9.74 \times e^{-5}$
2	4b	3	-6.92	-10.11	$8.42 \times e^{-6}$
3	4c	3	-8.35	-11.46	$7.61 \times e^{-6}$

Since an atomic-resolution structure of synthesized compounds bound to DNA is not yet available, there is no much experimental evidence regarding the orientation and binding modes of parent compounds **4(a-c)** when it is bound at the major groove. According to two criteria, prediction statistics and energy ranking, docking of isoxazole to DNA results in both energetically favorable binding mode and binding site **Figure(1)**.

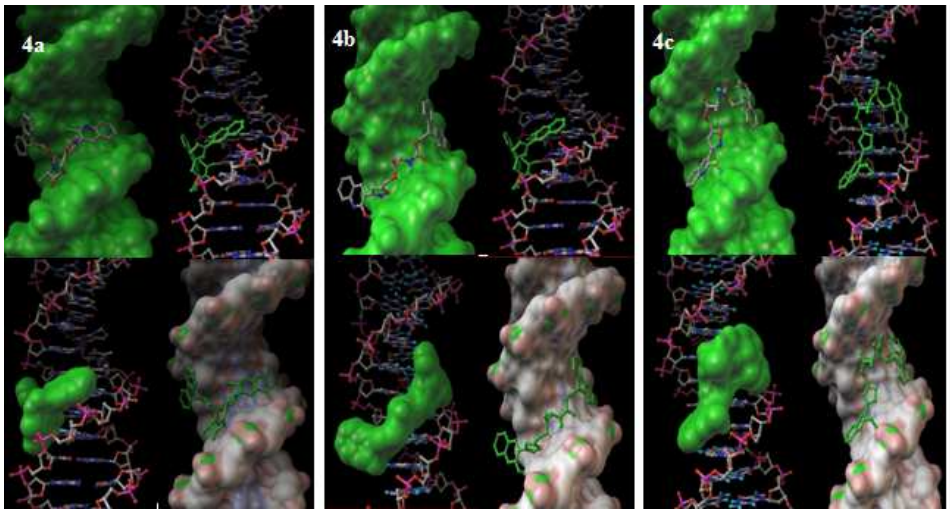


Figure-1. (a) Ball and Stick of DNA with the compound 4(a-c) (green ball and stick) and showing the hydrogen bond formation with bond length. (b) The synthesized compound (green ball and stick) is enfolded in the major groove of DNA showing interacting with chain A and chain B (MSMS-MOL). (c) Hydrogen bond between the compounds (stick and ball colored by green) and the N7 (DG16) residue of chain B of DNA and interacting with residues DA5, DA6, DT7, DT8, B7C9 of chain A and DG16 of chain B.

DNA binding studies

Primarily, the DNA binding were observed by the following parameters: (i) electrostatic interactions with the negative charged nucleic sugar–phosphate structure which are along the external DNA double helix and do not possess selectivity; (ii) binding interactions with two grooves of DNA double helix; and (iii) intercalation between the stacked base pairs of native DNA[39-40]. In comparison of compound **4(a-g)**, the compound **4a** shows good results for both DNA binding cleavage studies compared to other compounds**(Figure -2).**

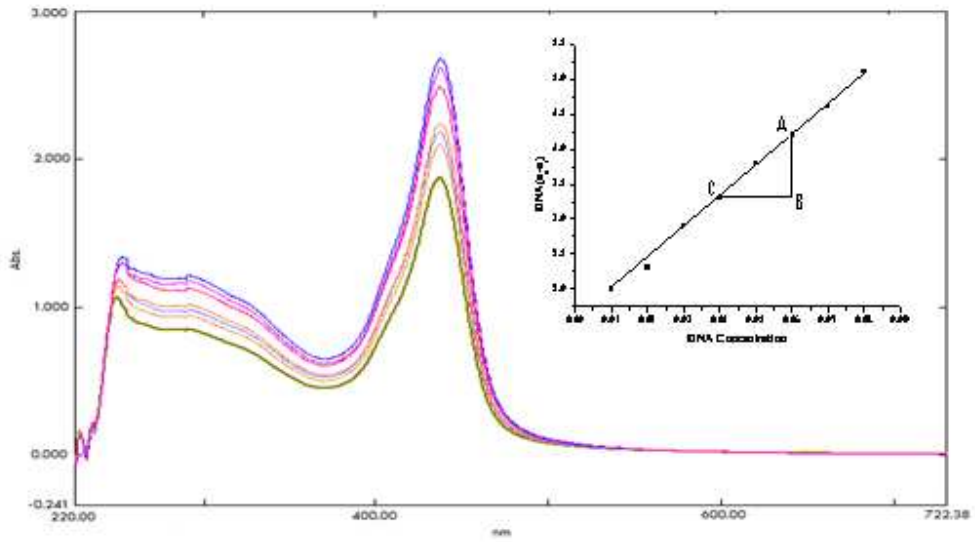


Figure-2. Absorption spectra of pyrazole in Tris–HCl buffer upon addition of DNA: = 0.5µM, [DNA] = 0–100µM. Showing variation in absorption with increase in concentration of [DNA]. Inner plot of [DNA]/ (εa − εf) versus [DNA] for titration of DNA.

The interaction of **4a** with DNA resulted in the decrease of absorption intensity accompanied by a shift towards higher wavelengths from 432nm. The addition of increasing higher concentration of DNA led to hypochromic and slight bathochromic (red shift) changes in its visible absorption spectra The DNA binding studies were characterized by absorbance maxima at 432 nm for quinoline isoxazole **4a**.Around 5–8% reduction (hypo-chromism) of absorption were observed at 432 nm peak maximum in the presence of an excess of calf thymus DNA. The lowest observation value observed in spectral changes (including red shift and hypochromicity) were used to evaluate intrinsic binding constant K_b, it observed 3.8×10^5 M^{-1} which are consistent with the intercalation of **4a** with DNA base pairs[41-42].

Viscosity Measurements

To further clarify, the interaction modes of the compounds **4(a-g)** with DNA were investigated by viscosity measurements. An increase in viscosity of native DNA is regarded as a diagnostic feature of an intercalation process[43-44]. The relative length increase (*L/L0*) of the compound formed between **4(a-g)** with DNA. It is evident that binding of these compounds increased the viscosity of DNA corresponding to an increase in the contour length of the DNA fragments. **Figure** (**3**).

The measured slopes of the plots 1.43 ± 0.03, for compound **4a-g** falls within 66 % of the slope of a theoretical curve for an idealized intercalation process (1 + 2*r*)[45]. On this basis, we calculated that intercalation of all molecule but 4a-d provoked an increase of 1.9 **Å** in the contour length of DNA. Since the sizes of these sonicated fragments were significantly greater than the persistence length, the estimated 1.9Å**,** lengthening is probably best regarded as a lower limit.

Thermal Denaturation

Other strong evidence for the intercalative binding of compounds into the double helix DNA was obtained from DNA melting studies. The intercalation of small molecules into the double helix is known to increase the DNA melting temperature (*Tm*), at which the double helix denatures into single stranded DNA owing to the increased

stability of the helix in the presence of an intercalator[46]. The molar extinction coefficient of DNA bases at 260 nm in the double helical form is much lesser than the single stranded form. Hence, melting of the helix leads to an increase in the absorbance at 432 nm. Thus, the helix to coil transition temperature can be determined by monitoring the absorbance of DNA at 432 nm as a function of temperature. The DNA melting studies were carried out with calf thymus DNA in the absence and presence of **4a-c** [1: 3 ratio of **4a-c** to DNA-c(P)]. The *Tm* for calf thymus DNA were 60±5°C

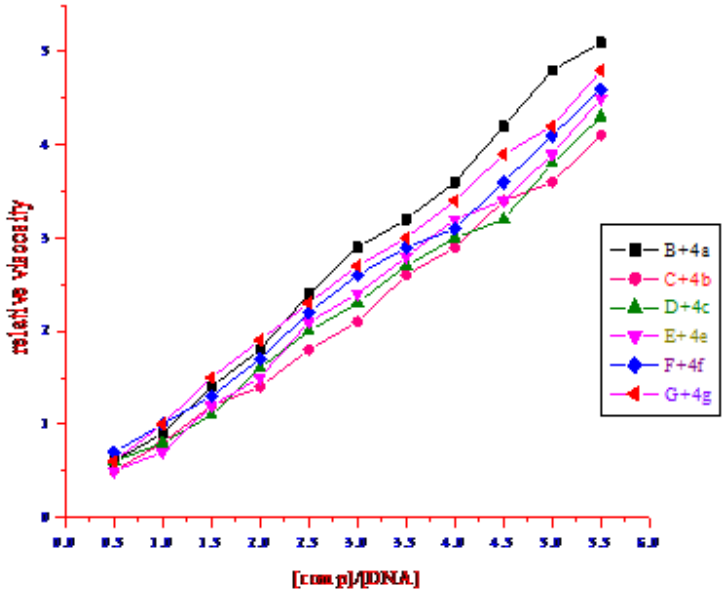


Figure-3: Plot shows effect of 4(a-g) on the viscosity of CT-DNA at 25 (±0.1°C). 4a = 0-100 µM and [DNA] = 50 µM.

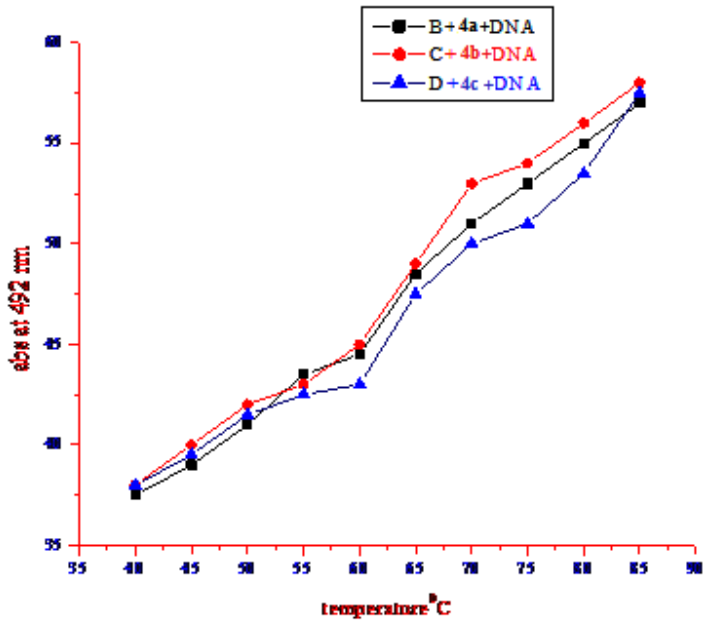


Figure-4: Melting curves of CT-DNA in absence and presence of compounds 4a in the absence of compounds, but in the presence of parent compounds 4(a-c) the *Tm* of CT DNA were increased by 5°C.

DNA Photo nuclease studies

We have also studied the photo-induced DNA cleavage activity of the compounds **4a-c** in the presence and absence of different additives using UV light at 365 nm. The faster-moving band corresponding to the native form of supercoiled circular DNA (SCDNA) and the slower-moving band being the open circular form (OC-DNA). Figure 5 shown that control experiments have suggested that untreated DNA does not show any cleavage even upon irradiation by UV light. Compounds 4a-c show significant nuclease activity in the absence of additives by converting SC DNA (Form I) to NC DNA (Form II) and linear form. The formation of singlet oxygen is further supported by the enhancement of percentage of SC DNA cleavage. lane 4 for 4a, lane 6 for 4b and lane 8 for 4c.

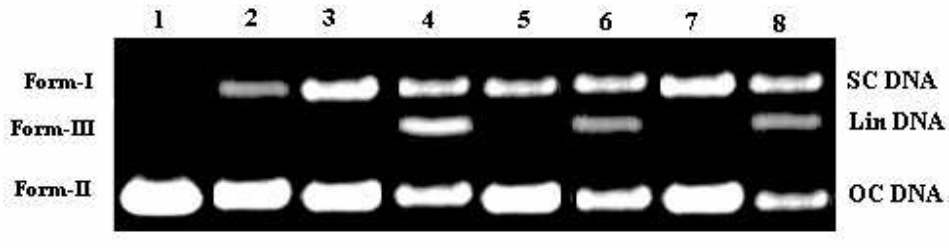


Figure-5: Effects of 6 at different concentrations (50-100 µmol/L) on the pUC 19 supercoiled DNA against $^{\cdot}OH$ generated by photolysis at 360 nm in presence of H_2O_2. Lane 1, Untreated DNA (control); lane 2, DNA + H_2O_2; lane 3, DNA + 4a (50 µmol/L); lane 4, DNA +4a (100 µmol/L); lane 5, DNA + 4b (50 µmol/L); lane 6, DNA + 4b (100 µmol/L); lane 7, DNA + 4c (50 µmol/L);lane 8, DNA + 4c (100 µmol/L).

Reaction that leads to formation of open circular DNA (form II) from the supercoiled (form I) over different concentrations of compound 4(a-c) (50-100 µM/L). The presence of the compounds 4a-c under investigation increases the DNA damage which has been particularly implicated in carcinogenesis with constant DNA concentration. These compounds are potential DNA cleaving agents at 37 ^{0}C.

CONCLUSION

We had developed a versatile and useful new access to different scaffold of biologically important newly synthesized quinoline isoxazole containing S/Se/Cl/OH. The compound **4a** and **4b** showed significant zone and minimum inhibitory concentration in antibacterial activity comp-ared to compound 4a-g. Compared to overall derivatives of quinoline isoxazole 4(a-g) compounds containing SH/SeH are more potent DNA binding and cleaving agent is reported. Information obtained from the present work will be helpful to the understanding of the mechanism of metal complexes with nucleic acids and should be useful in the development of potential probes of DNA structure and conformation.

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