DNA PHOTOCLEAVAGE AND ANTIMICROBIAL STUDIES ON NEW ISOXAZOLE DERIVATIVES

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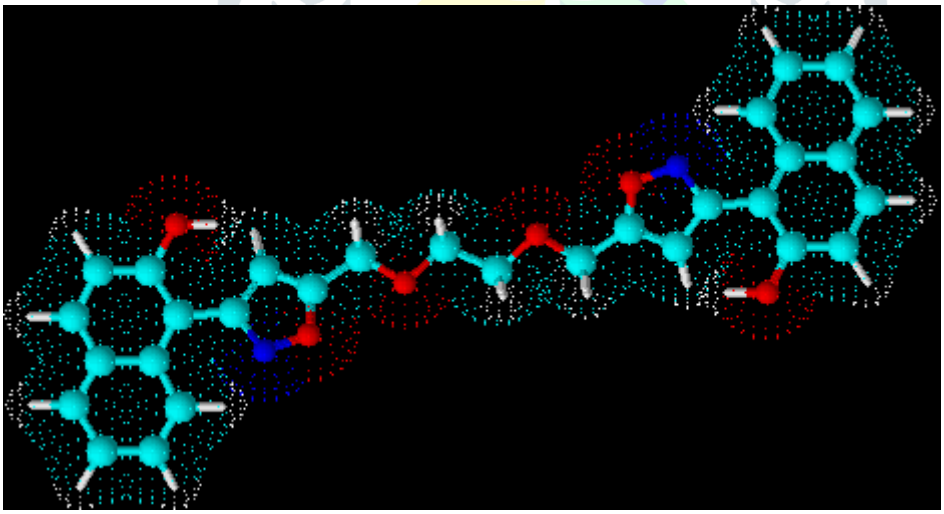
Abstract

A versatile method was adopted for the synthesis of isoxazole and its derivatives by using click chemistry. Isoxazole is further condensed with the ethylene-di-amine to obtain new *bis*-isoxazole moieties and characterized by IR, ^{1}H NMR and Mass spectra. These derivatives were auxiliary screened for antibacterial activity with succession of gram positive and gram negative bacteria which exhibited good results. The photo induced cleavage studies at 360 nm, exposed that the compounds possess photonuclease property against pUC19 DNA without inhibitors.

Index words: Antibacterial, inhibitors, isoxazole, DNAphotocleavage activity.



1. Introduction

The Isoxazole structure habitually appears as a significant component in biologically active natural compounds exhibiting a extensive range of pharmacological properties [1]. In particular, most of the fused isoxazole had importance in the medicinal and pharmaceutical chemistry [2-7]. 1,3-Dipolar cycloaddition is a valuable route for the construction of pharmacological active five-membered isoxazoles. A good yields of isoxazoles is achieved by 1,3-Dipolar cycloaddition of nitrile oxides with alkynes [8-9]. Isoxazole ring can serve as a bridge in carbohydrate chemistry such as isoxazole-linked steroidal glycol-conjugates and nucleoside derivatives[10-12].

The natural products such as ibotenic acid, muscimol and muscazone are excellent CNS active isoxazole compounds isolated from a mushroom called *Amanita muscaria* [13]. Another bioactive natural isoxazole triumferol is extracted from *Triumfetta rhomboidea*[14].

The carboxy-amido isoxazole scaffold was found to act as CB_2 receptor agonists[15]. Isoxazoles nucleii act as inhibitor of bromodomain and extra-C terminal domain (BET) proteins which controls the expression of genes which are critical for proper cell growth [16]. Isoxazoles nuclei also serves as potent and selective antagonism of anti-HIV activity [17].

Currently, bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant more quickly than new drugs are being found [18], thus, future research in antimicrobial therapy may focus on finding ways to overcome resistance to antimicrobials, or methods to treat infections with alternative means[19-20]. The pharmacological properties showed anti-convulsant activity by the thiohydantoins due to hydontain moiety [21-22]. Similarly many natural and synthetic products containing heterocyclic rings, such as isoxazole [23-25] were reported to possess various pharmacological activities.

In view of the above pharmaceutical and biologically importance and in continuation of our previous work [26-27], a new strategy was employed by using bis-isoxazole moiety as precursor without using high dilution conditions.

In support of the literature survey, the antibacterial activity and DNA photonuclease activity were screened to reveal and understand the mode of action. The DNA cleavage activity results obtained shows the DNA bond separated in the nuclease activity with our compound, which implies positive results can be gained with our newly synthesized bis-isoxazole compounds **k(1-6)**. We further evaluated the *in-vitro* antitumour activity of these synthesized compounds.

2. Experimental

2.1.Materials and methods

The reagents and solvents were purchased commerciallyfromMerckwith AR grade. All the solvents were purified by rota-evaporation before use. The uncorrected method is followed to evaluate the melting point by using capillary method. The characterization of the compounds were performed using FT-IR spectrophotometer, ^{1}H NMR and ESMS by mass spectra. The IR spectrum was recorded on a Shimadzu-FTIR(Model-8400S^1) Infrared spectrometer in KBr(Shimadzu,Kyoto,Japan). The ^{1}H NMR (400MHz) spectrum was recorded on a Bruker AMX 400 spectrometer (Bruker Optik, Ettlingen, Germany)at IISC,

Bangalore, Karnataka. The mass spectrum was obtained using Bruker Daltanics1200 m/z at Syngene International Limited. The elemental analysis is done by CHNOS analyzer. The thin layer chromatography study was done by precoated silica plates. The bacterial strains are procured from national laboratories, Pune, India.

2.1.1.Biological evaluation

In association with the National Chemical Laboratory (NCL), Pune, India, six clinical strains were gathered and out of which, 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 with 17% (v/v) glycerol in the Brain Heart Infusion (BHI). All the associated bacterial microorganisms were kept at 30^{o}C. The suspensions were then moved to luria-bertani broth and were cultured overnight at 37^{o}C with respect to pre-testing. Turbidity of the culture medium was set to match 0.5 McFarland standards, in order to prepare the inocula. The viability of the recipe was checked using the dilutions of 0.1% peptone (w/v) solution in sterile water inoculated on luria agar . Appraisal of antibacterial activity of the test samples was done by Antibacterial Assay the agar well diffusion method, using main reported protocol [28]. The reference antibacterial agent Ciprofloxacin (10 mg/mL) was loaded in the corresponding wells. Plates were then incubated at 37 ^{o}C for 24 h. Retarding zones formed on the medium was evaluated in millimetre after the completion of incubation.

As per the guidelines of Clinical and Laboratory Standards Institute (CLSI), USA, for newly synthesized compounds **k(1-6)**minimum inhibitory concentrations (MIC) were determined by micro dilution techniques in luria-bertani broth. With density of the medium being adjusted to a 0.5 McFarland turbidity standard colony forming units and by diluting 1:10 for the broth micro dilution procedure, the bacterial inoculates were prepared. MIC was estimated after 24 h of incubation, with themicrotiter plates being incubated at 37^{o}C. Potent antibacterial activity of our synthesized compounds was found by comparing with the standard drug.

2.1.2.DNA Photocleavage studies

Using the Agarose gel electrophoresis in Tris-HCl buffer (50 mM, pH 7.2) containing NaCl (50 mM), the extent of cleavage of super coiled (SC) pUC19 DNA (0.5 ml, 0.5 mg) to its nicked circular (NC) form was investigated. The reaction volumes were held in the caps of polyethylene micro centrifuge tubes, which were placed directly on the surface of a trans-illuminator (8000 mW/cm) at 360 nm. The samples were irradiated for 5 min at room temperature. Using monochromatic UV or visible light for irradiation of cleavage reactions of the 40 mM compound k(1-6)in 18 ml buffer was photo-irradiated for 1 h followed by addition of a 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.

Electrophoresis was carried out at 50 V for 3 h in tris-borate EDTA (TBE) buffer. Bands were visualized by UV light and were photographed to determine the extent of DNA cleavage from the intensities of the bands using UVITEC Gel Documentation System. Because of corrections 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 photoinduced DNA cleavage experiments and photographed under UV light at 360 nm.

2.2. Synthesis

The different substituted aldehydes (1equiv) were dissolved in butanol-water system at 3:1composition in a round bottom flask. Then NH_2OH**.**HCl (1.25 equiv) were added to form aldoxime then TsNCl**.**$3H_2O$ (1.25equiv) was added slowly followed by metal salt and metal turnings (copper sulphate (0.4 equiv) and copper metal).The above mixture was stirred for 30 min, later propagyl alcohol (1.25 equiv) was added. The complete reaction was stirred in room temperature for 4 h. The completed reaction was poured into the ice bathafter tartan by TLC was obtained.The ice cold mixture is then treated with ammonium hydroxide to confiscate the excess metal salts. Solid product was obtained by buckner filtration and dried under vaccum. The purification of compound was done by solvent extraction using suitable solvent to obtain 3-R-1,2-isoxazol-5-yl)methanol[m(1-6)] derivatives**.** The mixture of **m(1-6)** and ethylene diamine with 2:1 ratio was dissolved in DMF in a rb flask. Then refluxed at 60^oC for 3 to 4 h in presence of activated K_2CO_3 as catalyst. Thisreaction mixture was then quenched in ice, filtered and dried to obtain **k(1-6)**.

Compound 1k: 1-(5-{[3-(1,2-isoxazol-5-ylmethyl)ethylammine]methyl}-2(1,2-isoxazol-3-yl)benzene): Yield:-82%, Pale green colour solid,FT-IR (KBr)in cm^{-1}, 3240(-NH_2) 3122(=C-H), 2852(C-H str), 1616(C=N), 1571, 1527, 1444(Ar-C=C str), 1371(C-O), 1151(C-O-C)cm^{-1}. ^{1}H-NMR(DMSO-d_6), δ(in ppm) 2.5(s,4H, - CH_2), 3.5(s, 4H, -CH_2), 5.2(bs, 2H,-NH), 6.1(s, 2H, Ar-H), 7.2(d, 2H, Ar-H), 7.4(dd, 4H, Ar-H), 7.5(d, 4H, Ar-H).ESMS *(m/z)*: 375.1 $(M)^+$.

Compound 2k: 1-(5-{[3-(1,2-isoxazol-5-ylmethyl) ethylamine]methyl}-2(1,2-isoxazol-3-yl)phenol) :Yield:-84%, green solid, FT-IR (KBr) in cm^{-1}, 3428(Ar-OH), 3242(-NH str), 3124(=C-H), 2918 (C-H str), 1612(C=N), 1570, 1527, 1454(Ar-C=C str), 1370(C-O), 1151(C-O-C)cm^{-1}. ^{1}H-NMR(DMSO-d_6), δ(in ppm) 2.6(d, 4H, -CH_2), 3.6(s, 4H,-CH_2-), 5.2(bs,2H,-OH), 6.1(s, 2H, Ar-H), 7.3(d, 2H, Ar-H) 7.4(d, 2H, Ar-H), 7.5(d, 2H, Ar-H), 7.8(d, 2H, -CH_2), 8.2(bs, 2H, -NH).ESMS *(m/z)*: 407.1 $(M)^+$.

Compound 3k : 1-(5-{[3-(1,2-isoxazol-5-ylmethyl) ethylamine]methyl}-2(1,2-isoxazol-3-yl)-chloro-benzene): Yield-75%, yellow solid, FT-IR (KBr) in cm^{-1}, 3240(-NH str), 3128(=C-H), 2918(C-H str), 1614(C=N), 1572, 1522, 1442(Ar-C=C str), 1374(C-O), 1151(C-O-C),724(C-Cl)cm^{-1}. ^{1}H-NMR(DMSO-d_6), δ(in ppm) 2.5(d, 4H, -CH_2), 3.4(s, 4H,-CH_2-), 6.2(s, 2H, Ar-H), 7.3(t, 2H, Ar-H), 7.5(t, 2H, Ar-H), 7.7(d, 2H, Ar-H), 7.9(d, 2H, Ar-H), 8.2(bs, 2H, -NH).ESMS *(m/z)*: 443.1,445.1$(M)^+$.

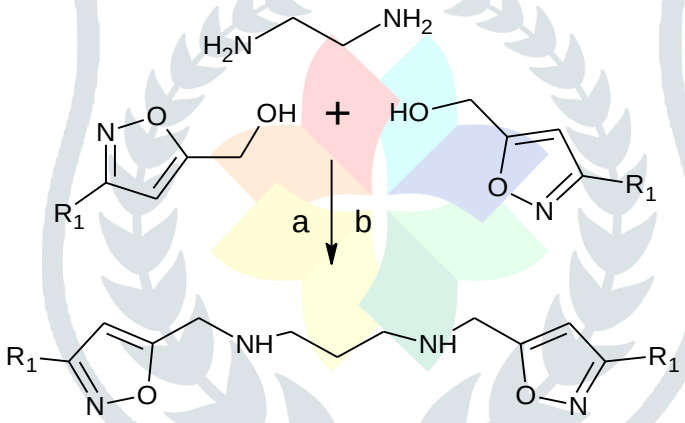
Compound 4k : 1-(5-{[3-(1,2-isoxazol-5-ylmethyl) ethylamine]methyl}-2(1,2-isoxazol-3-yl)-hydoxy-napthalene): Yield-74%, yellowish green solid, FT-IR (KBr) in cm^{-1}, 3428(Ar-OH), 3248(-NH str), 3112(=C-H), 2911(C-H str), 1610(C=N), 1571, 1527, 1447 (Ar-C=C str), 1375(C-O), 1151(C-O-C)cm^{-1}. ^{1}H-NMR(DMSO-d_6), δ(in ppm) 2.6(t, 4H, -CH_2), 3.4(s, 4H,-CH_2-), 5.2(bs,2H,-OH), 6.1(s, 2H, Ar-H), 7.3(d, 2H, Ar-H), 7.4(d, 2H, Ar-H), 7.5(d, 2H, Ar-H), 7.6(d, 4H, Ar-H), 7.8(d, 2H, -CH_2), 8.2(bs, 2H, -NH).ESMS*(m/z)*: 507.2 $(M)^+$.

Compound 5k: 1-(5-{[3-(1,2-isoxazol-5-ylmethyl)ethylammine]methyl}-2(1,2-isoxazol-3-yl)-3-hydroxy-4-methoxybenzene): Yield-76%, light brown solid, FT-IR (KBr) in cm^{-1}, 3424(Ar-OH), 3244(-NH str), 3038(=C-H), 2919(C-H str), 1617(C=N), 1571, 1527, 1454(Ar-C=C str), 1375(C-O), 1151(C-O-C). ^{1}H-NMR(DMSO-d_6), δ(in ppm) 2.4(t, 4H, -CH_2), 3.1(s, 4H,-CH_2-), 3.6(s, 6H, -CH_3) 5.0(bs,2H,-OH), 6.2(s, 2H, Ar-H), 7.4(d, 2H, Ar-H), 7.6(s, 2H, Ar-H), 7.8(d, 2H, Ar-H), 8.0(bs, 2H, -NH).ESMS (*m/z*):467.1 $(M)^+$.

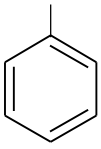
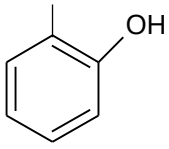
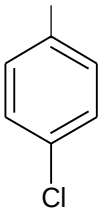
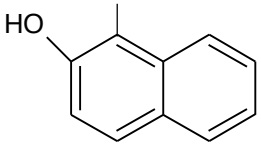
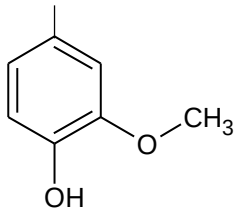
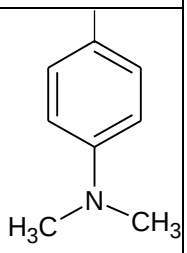
Compound 6k:1-(5-{[3-(1,2-isoxazol-5-ylmethyl)ethylammine]methyl}-2(1,2-isoxazol-3-yl)-4-4-di-methyl-amino benzene): Yield-80%, yellowish brown solid, FT-IR (KBr) in cm^{-1}, 3241(-NH str), 3120(=C-H), 2918(C-H str), 1615(C=N), 1571, 1527, 1446(Ar-C=C str), 1376(C-O), 1154(C-O-C). ^{1}H-NMR(DMSO-d_6), δ (in ppm) 2.4(t, 4H, -CH_2), 2.5(s, 12H,-4CH_3) 3.6(s, 4H,-CH_2-), 5.0(bs, 2H, -NH), 6.2(s, 2H, Ar-H), 7.4(d, 4H, Ar-H). 7.8(d, 4H, Ar-H). ESMS *(m/z)*:461.2 $(M)^+$.

3. Results and discussion

Huisgen's classic 1,3-dipolar cycloaddition process was made use for the synthesis of isoxazole using its stepwise variant. Oxidative coupling products that are often observed are prevented by the addition of the catalyst under room temperature condition with a slight excess of chloroamine-T. This will result in the rapid formation of an array of isoxazole compounds.



Scheme:Synthesis of compounds k(1-6);a) Dried DMF b) activated K_2CO_3 with 70°C reflux.

COMPOUND	1k	2k	3k	4k	5k	6k
-R_1						

Our protocol establishes that the heterocyclic aldehydes **k(1-6)** were first converted into corresponding aldoxime through the reaction with hydroxylamine.

Table 1: The physical characterization data of compounds k(1-6)

Comp. No.	Product M.P(in ^{0}C)	Elementary Analysis(found)%				
		C	H	N	O	C
1k	$C_{22}H_{22}N_4O_2$ (88-90 ^{0}C)	70.57 (70.60)	5.92 (5.94)	14.96 (14.94)	8.55 (8.59)	-
2k	$C_{22}H_{22}N_4O_4$ (126-128 ^{0}C)	65.01 (65.04)	5.46 (5.49)	13.78 (13.81)	15.75 (15.76)	-
3k	$C_{22}H_{20}Cl_2N_4O_2$ (136-138 ^{0}C)	59.60 (59.64)	4.55 (4.58)	12.64 (12.68)	7.22 (7.25)	15.99 (15.96)
4k	$C_{30}H_{26}N_4O_4$ (142-144 ^{0}C)	71.13 (71.16)	5.17 (5.19)	11.06 (11.10)	12.63 (12.67)	-
5k	$C_{24}H_{26}N_4O_6$ (158-160 ^{0}C)	61.79 (61.83)	5.62 (5.64)	12.01 (12.06)	20.58 (20.62)	-
6k	$C_{26}H_{32}N_6O_2$ (148-150 ^{0}C)	67.80 (67.84)	7.00 (7.04)	18.25 (18.29)	6.95 (6.94)	-

The reaction with hydroxylamine and heterocyclic aldehydes (1-6) was first converted into corresponding aldoxime. Using chloroamine-T trihydrate, which acts as both a halogenating agent and a base The aldoxime was transformed to analogous nitrile oxide **k(1-6)**.The isolation and conduct of potentially harmful and unstable hydroximoyl chlorides is avoided. Thus, nitrile oxide reacted only with the terminal propagyl alcohol to form derivatives**k(1-6)**.This procedure offers good yields of cyclization products with short reaction times and simple purification. The characteristic data of compound is given in **Table 1**. The synthesized derivatives of isoxazoles **k(1-6)**were well characterised by using H^1NMR FTIR and mass spectroscopy. The spectrum shown singlet and doublet, triplet and double doubled for particular corresponding peak for particular proton. The mass spectrum showed mass isolation peak for respective compounds and isotopic peak for the isotopes in mass spectrum.

3.2. Spectral analysis

Compound **1k** consists of 22 protons in the 7 different types of moeity. The peak at 2.5ppm corresponds to aliphatic methylene protons(s,4H) the protons attached to isoxazole shows a NMR signal at 3.5ppm(s,4H). Aromatic protons of isoxazole ring is given and NMR signal at 6.1ppm(s,2H) and the aromatic protons of benzene ring are of three types and they show NMR peaks at 7.2ppm(d,2H), 7.4ppm (dd,4H) and 7.5ppm(d,2H). The broad singlet peak at 5.2ppm(bs,2H) corresponds to -NH protons of ethylenediamine.

The compound shows an IR stretching frequency at 3240-3250cm^{-1} for the NH Bond and carbon-carbon double bond stretching frequency at 1440-1445 cm^{-1} and C=N bond stretching frequency of isoxazole ring at 1610-1622 cm^{-1}. An IR band 1151 cm^{-1}shows the C-O stretch of isoxazole ring. The molecular ionization peak at 375.1 with the positive mode was observed for **1k**.

Compound **2k**consists of 22 protons and due to the presence of phenolic -OH the NMR signal of the -NH protons is observed at 8.2ppm(bs,2H) and a sharp peak at 5.2ppm(s,2H) confirm the phenolic -OH group. Due to the presence of -OH group on benzene ring all the protons are chemically non-equivalent and they show individual peak at 7.5ppm(d,2H),7.8ppm(dd,2H), 7.3ppm(dd,2H) and 7.4ppm(d,2H). The compound

2k shows and IR differentiating band at frequency 3428cm^{-1}confirming the presence of phenolic -OH Bond stretch. This IR band is not observed in compound **1k**. The mass of the compound corresponds to molecular Ion peak at 407.1 with positive ionization.

Compound **3k** has 20 protons and the main differentiating NMR peaks at 7.3ppm (d,2H), 7.5ppm(d,2H), 7.7ppm(d,2H) and 7.9ppm(d,2H)for four sets of aromatic protons. Absence of -OH Proton peak differentiates **3k** from compound **2k**.An intense IR stretching band at a frequency 724cm^{-1} shows the C-Cl stretch which was not shown by **1k** and **2k**.A molecular Ion peak in mass spectra is observed at 443.1and also a peek at 445.1 in and intensity ratio of 3:1 confirmingthe isotopic peaks corresponds to $^{35}Cl$and^{37}Cl.

Compound **4k** has 26 protons and additional protons are of naphtholic ring. A NMR peak at 5.2ppm(s,2H) indicate the naphtholic -OH protons and a broad singlet peak at 8.2ppm(bs,2H) for -NH protons in the downfield due to the presence of -OH.

An IR stretching band at frequency 3428cm^{-1} confirms the presence of naphtholic -OH Bond which differentiates **4k** from compound **3k**.Compound **5k**out of 26 protons, the NMR signal at 3.6ppm(s,6H) in a field confirms the presence of methoxy protons and a peak at 5.0ppm(s,2H)confirms the phenolic -OH protons and abroad singlet peak at 8.0ppm(bs,2H) confirms the NH protons coupled with OH and appear in downfield.

3.2.Biological activity

3.2.1.Antibacterial activity

The obtained Inhibition zone values for *S.aureus*is 15.90 and 16.90 for compounds **1k** and **2k** respectively.Whereas in *S.pyogenes*shown good bacterial activity for **1k** and **2k**. whereas for **3k,4k,5k**and **6k** shown almost good results for the activity for *S.pyogenes*. The antibacterial activity for the *pseudomonous aeraginosa* shown good results for **1k,3k** and **4k**. The gram negative bacteria *bacillius subtillis*shows better results **1k,3k** and **4k**. whereas **2k,5k** and **6k** showed moderate results with *B.subtillis.*The standard drug ciprofloxin shown 24.70 and 22.40 for both gram positive and gram negative bacteria.The overall comparison studies of all bacteria with different derivatives show better results when compared with the standard drug.The **2k** and **3k** showed better results in *s.pyogenes* due to the presence of –OH group and –Cl group in the molecule. Whereas **4k,5k** and **6k** also consists of –OH group but due to hindrance in the moiety, the molecule activity may vary.

MIC values for *S.aureus*of different compounds were as follows, 9.0 mg/mL for compound **2k** and 8.9 mg/mL for compound **4k**. The assessment of results of antibacterial activity of novel synthesized compounds from **k(1-6)**, compounds **2k, 3k**containing -OH and -Cl showed a significant synchronizing effect on bacterial strains. The zone of inhibition of the bacterial colonies are depicted in **Table 2**.

Table 2: Shows zone of inhibition of different bacteria for compounds k(1-6).

Diameter of zone of inhibition (mm) Clinical strains				
Compounds	***S.pyogenes***	***S.aureus***	***P.aeruginosa***	***B.subtillis***
1k	18.20 ± 0.10	15.90 ± 0.10	16.30 ± 0.10	17.40±0.10
2k	18.90 ± 0.10	16.90 ± 0.10	15.60 ± 0.10	15.90±0.10
3k	17.60± 0.10	16.10± 0.10	16.60± 0.10	16.10±0.10
4k	17.80± 0.10	15.80± 0.10	16.10± 0.10	16.10±0.10
5k	16.90± 0.10	15.20± 0.10	15.90± 0.10	15.70±0.10
6k	17.10± 0.10	15.50± 0.10	15.50± 0.10	14.90±0.10
Ciproflaxin	23.80 ± 0.10	24.70 ± 0.10	23.10 ± 0.10	22.40±0.10

Compound **5k** demonstrated antibacterial activity against all the strains of bacteria. But most significant activity was seen on Gram-positive bacteria *S.aureus*(15.20mm) and *S.pyogenes*(16.90 mm). Among the newly synthesized compounds, compound **3k** containing -OH component exhibited a more potent bactericidal agent against *S.aeruginosa*(16.60 mm) and *B.subtilis*– NCIM- 2010 (16.10 mm). Compound **4k** containing chloro substituent showed a meager antimicrobial property against all the pathogenic strains of bacteria.The actual mechanism of antibacterial activity is due to biocontrolling.Whereas the cell walls of Gram-negative bacteria were impermeable to drug constituents since they have an outer phospholipidic membrane carrying the structural lipo-polysaccharide components.

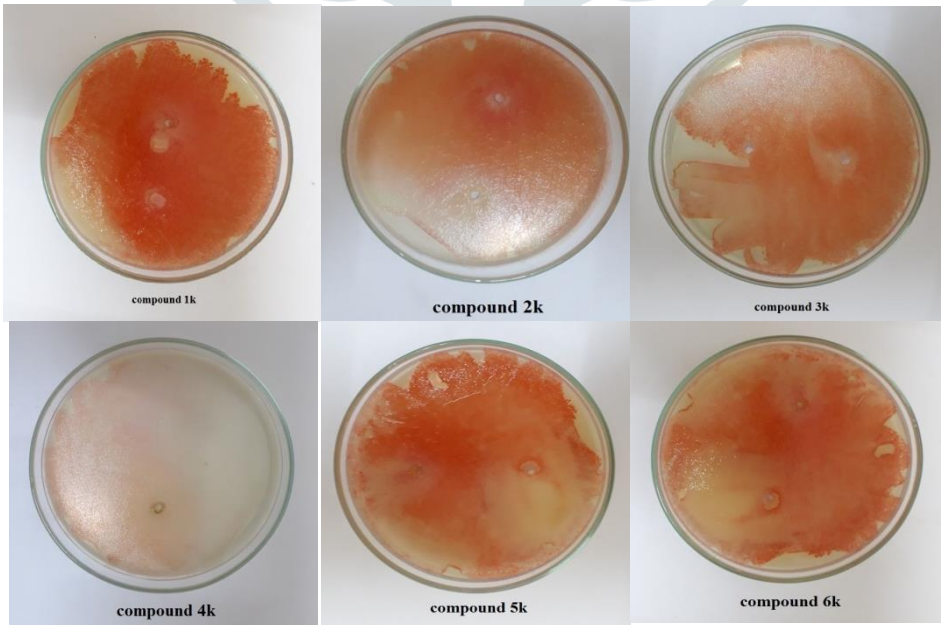


Fig: 1: Zone of inhibition of gram+ve and gram –ve bacterial with k(1-6)

The minimum inhibitory activity is observed for Gram-positive bacteria *S.aureus.* The zone of inhibitory activity is significantin the case of Gram-negative *P.aeruginosa*,because of a multilayered phospholipidic membrane carrying the structural lipo-polysaccharide components.

The **2k,3k** and **6k** shown MIC values for inhibition for s.pyogenes. Whereas**3k** and **4k** values for *S.aureus*. In *P.aeruginosa* the **1k,2k** and **6k** shown the better MIC values compared to **3k,4k** ans **5k** shown meager results for MIC. The gram negative bacteria *bacillus subtilis* shown meager for all the derivatives in MIC values. Hence the molecules which contain –OH, -Cland $–OCH_3$ shown good results compared to simple aromatic compound.

Table 3: MIC (mg/mL) values of the synthesized compounds k(1-6)

Bacterial strains Minimal inhibitory concentrations (MIC)-(mg/mL)				
Compounds	***S.pyogenes***	***S.aureus***	***P.aeruginosa***	***B.subtilis***
1k	10.9	8.6	10.2	12.5
2k	10.9	9.0	10.1	12.9
3k	12.1	10.1	11.6	11.1
4k	10.3	10.4	11.9	11.6
5k	10.2	8.4	11.5	11.5
6k	10.9	11.2	11.9	11.9
Ciprofloxacin	7.45	7.65	7.5	7.25

3.2.2.DNA cleavage studies

The photo-induced DNA photocleavage activities of the newly synthesized bis-isoxazole compounds were studied. Enhancement of percent SC DNA further supports the formation of singlet oxygen cleavage, studied by using UV light at 365 nm. These results clearly indicate that compounds at different concentrations are solely responsible for nuclease activity through a singlet oxygen mediated mechanism.

The compounds were tested for DNA cleavage under hydrolytic conditions, and a concentration dependent cleavage was observed. Based on the binding results photolysis activity of compounds k(1-6) was selected. Reaction that leads to formation of open circular DNA (form II) from the supercoiled (form I) over various concentrations of compounds k(1-6) (50 μM/L) and constant DNA concentration was followed for different concentrations at 37 ºC.

LANE 1 2 3 4 5 6 7 8

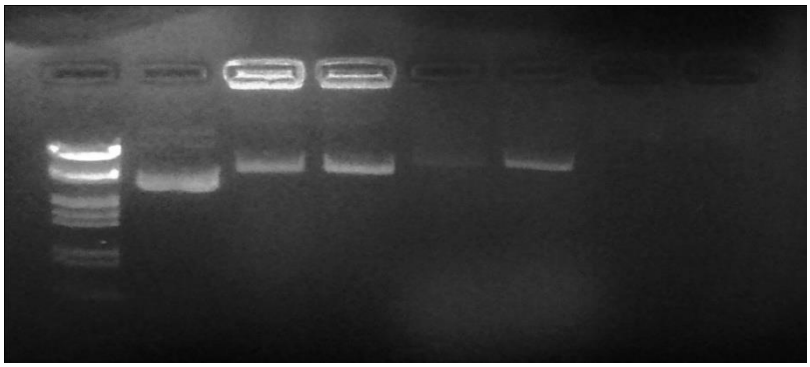


Fig 2:Effects of six at various concentrations (50µmol/L) on the pUC 19 supercoiled DNA against ˙OH generated by photolysis at 360 nm in presence of H_2O_2.

And following observations were made, Lane 1, Untreated DNA (control); lane 2, DNA + H_2O_2; lane 3, DNA + **1k** (50µmol/L); lane 4, DNA +**2k** (50 µmol/L); lane 5, DNA + **3k** (50 µmol/L); lane 6, DNA + **4k** (50 µmol/L); lane 7, DNA + **5k** (50 µmol/L): lane 8, DNA + **6k** (50 µmol/L).

4. Conclusion

The result of investigation confirms the structures of synthesized compounds **k(1-6)**by analytical techniques. The synthesised compounds showed inhibition in the bacterial growth with inhibitory concentration. The interactions of the compounds with DNA are of major biochemical importance. The evaluation of compounds with DNA interaction studies showed high potential as DNA photo cleaving agent. The DNA cleavage activity is evidence for the anti-carcinogenic activity. We can conclude that our newly synthesized compounds have more importance in medicinal field.

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