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Research Article

DNA INTERACTION STUDIES, ANTIMICROBIAL AND ANTHELMINTIC ACTIVITY OF SULFONAMIDES OF 1, 3-DIOXOLANE AND 3,4-DIHYDROQUINOLIN-2(1H)-ONE ANALOGUES: SYNTHESIS AND CHARACTERIZATION

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ABSTRACT

A series of sulfonamide group containing compounds were synthesized by the reaction of 2-dimethylaminomethyl-1,3-dioxolane, **I** and 7-hydroxy-3,4-dihydroquinolin-2(1H)-one, **II** with appropriate substituted sulfonyl chlorides, **IIIa-e**. The structural elucidation of these compounds is based on IR, ^{1}H-NMR, mass spectral data and elemental analysis. The synthesized compounds were tested for antimicrobial activity by disc diffusion method. Anthelmintic activity of the compounds has been tested on earthworms and few of the compounds show moderate activity. The DNA binding of the compounds **IVa** and **Va** with CT-DNA has been performed with absorption spectroscopy, which reveals that both the compounds show less binding propensity towards CT-DNA. Also the nuclease activity of complexes **IVa** and **Va** with plasmid DNA (P^{UC} 19) was studied using agarose gel electrophoresis. The compound **Va** can act as effective DNA cleaving agent when compared to compound **IVa** resulting in the nicked form of DNA under physiological conditions. The gel was run both in the absence and presence of the oxidizing agent.

Keywords: 3,4-dihydroquinolin-2(1H)-one, antibacterial activity, anthelmintic activity and DNA interaction.

INTRODUCTION

The need of new antimicrobial agents is justified because more microorganisms are being resistance to the currently available antibacterial drugs and this is bringing alarming threat to public health and causing growing concern among people across the globe. At the same time as the old antibiotics are losing their effectiveness, the supply of new drugs is drying up. Worldwide researchers are trying to synthesize new drugs with better pharmacokinetic and dynamic properties with less adverse effects.

The sulfonamide drugs were the first effective chemotherapeutic agents to be employed systemically for the prevention and cure of bacterial infection in human beings [1-4**]**. Due to this reason, sulfonamides occupy a unique position in the drug industry. The sulfonyl group plays a very important role as key constituent of a number of biologically active molecules.

At present, over 30 drugs containing the sulfonamide moiety are used clinically, as therapeutic agents and for the treatment of bacterial and viral infections. Sulfonamides are also diuretics, anticonvulsants and hypoglycemic agents as well as protease inhibitors. Arylsulfonyl substituents have been used as effective protecting groups for oxygen and nitrogen functionalities.

1,3 – dioxolane was found to be devoid of hypnotic activity but, in a detailed pharmacological study, it was found that some of these compounds are potent spasmolytic agents; they also exhibit antihistaminic activity.

Quinolin-2(1*H*)-ones are ubiquitous structural motifs that can be found in many naturally and non-naturally occurring compounds [5]. Many of these heterocycles possess interesting biological properties and have been developed as drugs that are antibiotics [6], HIV-1 reverse transcriptase inhibitors [7]. Dihydroquinoline derivatives have also received substantial attention due to their potential biological activities arising from their antioxidative properties [8, 9] as well as their usefulness as precursors of some other biologically active compounds [10, 11].

However, little is known about substituted 1,3-dioxolane and dihydroquinolin-2(1H)-one with sulphonamido group. Therefore, in the present work, we have synthesized the compounds containing sulphonamido group incorporated with 2-dimethylaminomethyl-1,3-dioxolane and 7-hydroxy-3,4-dihydroquinolin-2(1H)-one to get good biodynamic leads.

MATERIALS AND METHODS

All the solvents and chemicals are of Analytical grade and were purchased from commercial sources. Supercoiled (SC) pUC19 DNA was purchased from Bangalore Genei (India). Calf thymus DNA (CT DNA) was purchased from Sigma Aldrich, Germany.

EXPERIMENTAL

All the melting points were determined with open capillary tube by Cintex melting point apparatus and were uncorrected. IR spectra were recorded on a Shimadzu FTIR 8401 instrument by nujol method. ^{1}H-NMR spectra were recorded at 300 MHz on Bruker DRX 300 NMR spectrometer using TMS as internal standard and $CDCl_3$ as solvent. Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer. The elemental analysis (C, H, N, S) of compounds were carried out by Perkin-Elmer 2400 CHN elemental analyzer. The reaction was followed up and purity of the product was carried out on pre-coated TLC plates (silica gel G plates, Merck) using ethyl acetate/chloroform (8:2, v/v) solvent system and visualizing the spots in ultraviolet light. UV–Vis spectra were recorded on a Hitachi U-3900 spectrophotometer.

General procedure for the preparation of N-((1,3-dioxolan-2-yl)methyl)-N-benzenesulfonamide, IVa-IVe

To a solution of 2-dimethylaminomethyl-1,3-dioxolane (0.1171 g, 1mmol) in dichloromethane (10 ml), was added respective sulfonyl chloride **IIIa-e** (1.1mmol) and triethylamine (TEA) (2 mmol). The reaction mixture was then stirred continuously for 3 hours. The progress of the reaction was monitored by thin layer chromatography (TLC) by time to time. After completion of the reaction, the contents were washed with water and brine solution, dried over anhydrous sodium sulphate and recrystallized from ethanol.

N-((1,3-dioxolan-2-yl)methyl)-4-chloro-N-methylbenzenesulfonamide, IVa

Yield:82 %; Mp.: 58-61; FTIR (nujol) [cm^{-1}]: 2845 (N-CH_{str}), 2359 (-O-CH-O_{str}), 1168 (-SO_2-N_{str}), 1036 (dioxalane moiety); ^{1}H-NMR (300

MHz, $CDCl_3$): 2.42 (s, 3H, N-CH_3), 3.21 (d, 2H, N-CH_2-), 3.95 (t, 4H, -O-CH_2-CH_2-O-), 5.10 (t, 1H, -O-CH-O-), 7.32-7.66 (m, Ar-H); MS [m/z]: 292 (M+1); Anal. Calcd. for $C_{11}H_{14}ClNO_4S$: C, 45.28; H, 4.84; N, 4.80; S, 10.99. Found: C, 45.29; H, 4.85; N, 4.79; S, 11.01.

N-((1,3-dioxolan-2-yl)methyl)-N-4-dimethylbenzenesulfonamide, IVb

Yield: 73 %; Mp. 62-65; FTIR (nujol) [cm^{-1}]: 2359 (-O-CH-O_{str}), 1594 (N-CH_{str}), 1166 (-SO_2-N_{str}), 1037 (dioxalane moiety); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.43 (s, 3H, N-CH_3), 3.19 (d, 2H, N-CH_2-), 3.95 (t, 4H, -O-CH_2-CH_2-O-), 5.04 (t, 1H, -O-CH-O-), 7.32-7.66 (m, Ar-H); MS [m/z]: 272 (M+1); Anal. Calcd. for $C_{12}H_{17}NO_4S$: C, 63.49; H, 5.89; N, 3.90; S, 8.92. Found: C, 63.51; H, 5.90; N, 3.89; S, 8.95.

N-((1,3-dioxolan-2-yl)methyl)-N-methyl-2-nitrobenzenesulfonamide, IVc

Yield: 69 %; Mp. 61-64; FTIR (nujol) [cm^{-1}]: 2359 (-O-CH-O_{str}), 1167 (-SO_2-N_{str}), 1541 (N-CH_{str}), 1347 (Ar-N_{str}); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.43 (s, 3H, N-CH_3), 3.13 (d, 2H, N-CH_2-), 3.95 (t, 4H, -O-CH_2-CH_2-O-), 5.08 (t, 1H, -O-CH-O-), 7.27-7.69 (m, Ar-H); MS [m/z]: 304 (M+1); Anal. Calcd. for $C_{11}H_{14}N_2O_6S$: C, 43.70; H, 4.67; N, 9.27; S, 10.61. Found: C, 43.73; H, 4.68; N, 9.26; S, 10.62.

N-((1,3-dioxolan-2-yl)methyl)-2,5-dichloro-N-methylbenzenesulfonamide, IVd

Yield: 80 %; Mp. 65-68; FTIR (nujol) [cm^{-1}]: 2359 (-O-CH-O_{str}), 1155 (-SO_2-N_{str}), 1098 (Ar-1,4-Cl_{str}); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.80 (s, 3H, N-CH_3), 3.47 (d, 2H, N-CH_2-), 3.88-3.96 (t, 4H, -O-CH_2-CH_2-O-), 5.07 (t, 1H, -O-CH-O-), 7.26-8.10 (m, Ar-H); MS [m/z]: 327 (M+1); Anal. Calcd. for $C_{11}H_{13}ClNO_4S$: C, 40.05; H, 4.02; N, 4.29; S, 9.83. Found: C, 40.08; H, 4.03; N, 4.28; S, 9.85.

N-((1,3-dioxolan-2-yl)methyl)-3-tert-butyl-N-methylbenzenesulfonamide, IVe

Yield: 79 %; Mp. 63-67; FTIR (nujol) [cm^{-1}]: 2359 (-O-CH-O_{str}), 1159 (-SO_2-N_{str}), 1376 (-C(CH_3)$_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.88 (s, 3H, N-CH_3), 3.21 (d, 2H, N-CH_2-), 3.85-3.96 (t, 4H, -O-CH_2-CH_2-O-), 5.05 (t, 1H, -O-CH-O-), 7.26-7.72 (m, Ar-H); MS [m/z]: 314 (M+1); Anal. Calcd. for $C_{15}H_{23}NO_4$: C, 57.48; H, 7.40; N, 4.47; S, 10.23. Found: C, 57.50; H, 7.41; N, 4.49; S, 10.26.

General procedure for the preparation of 7-hydroxy-(phenylsulfonyl)-3,4-dihydroquinolin-2[1H]-one (Va-Ve)

A mixture of 7-hydroxy-3,4-dihydroquinolin-2(1H)-one (1 mmol) and respective sulfonyl chloride **IIIa-e** (1.1 mmol) and triethylamine (2 mmol) in dichloromethane solution (10 ml). The reaction mixture was continuously stirred for 3 h. Progress of the reaction was monitored by TLC analysis using silica gel as adsorbent and ethyl acetate-hexane (1:2) mixture as eluent. Product was isolated from the reaction mixture by separating triethyl amine hydrochloride. The residue was purified by washing with water followed by recrystallization from ethanol.

1-(4-chlorophenylsulfonyl)-7-hydroxy-3,4-dihydroquinolin-2(1H)-one, Va

Yield: 74 %; Mp. 127-131; FTIR (nujol) [cm^{-1}]: 1681 (-N-C=O_{str}), 1189 (-SO_2-N_{str}), 1079 (Ar-OH_{str}). ^{1}H-NMR (300 MHz, $CDCl_3$): 2.63 (t, 2H, -CH_2-), 2.94 (t, 2H, -CH_2-), 6.52-7.08 (d, 2H,Ar-H), 7.26-7.81 (s, 4H, Ar-H), 8.41 (s, 1H, Ar-OH); MS: m/z 338 (M+1); Anal. Calcd. for $C_{15}H_{12}ClNO_4S$: C, 53.34; H, 3.58; N, 4.15; S, 9.49. Found: C, 53.36; H, 3.60; N, 4.16; S, 9.50.

7-hydroxy-1-tosyl-3,4-dihydroquinolin-2(1H)-one, Vb

Yield: 70 %; Mp. 121-125; FTIR (nujol) [cm^{-1}]: 1681 (-N-C=O_{str}), 1192 (-SO_2-N_{str}), 1080 (Ar-OH_{str}), 367 (Ar-CH_3); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.44 (s, 3H, -CH_3), 2.51 (t, 2H, -CH_2-), 2.80 (t, 2H,-CH_2-), 6.24-6.88 (d, 2H, Ar-H), 7.43-7.71 (s, 4H, Ar-H), 9.90 (s, 1H, Ar-OH); MS [m/z]: 317 (M+1); Anal. Calcd. for $C_{16}H_{15}NO_4S$: C, 60.55; H, 4.76; N, 4.41; S, 10.10. Found: C, 60.56; H, 4.77; N, 4.40; S, 10.12.

7-hydroxy-1-(2-nitrophenylsulfonyl)-3,4-dihydroquinolin-2(1H)-one, Vc

Yield: 73 %; Mp. 131-135; FTIR (nujol) [cm^{-1}]: 1681 (-N-C=O_{str}), 1170 (-SO_2-N_{str}), 1078 (Ar-OH_{str}), 855 (Ar-N_{str}); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.44 (t, 2H, -CH_2-), 2.86 (t, 2H, -CH_2-), 6.28-6.77 (d, 2H, Ar-H), 6.84-7.23 (s, 4H, Ar-H), 9.90 (s, 1H, Ar-OH); MS [m/z]: 348 (M+1); Anal. Calcd. for $C_{15}H_{12}N_2O_6S$: C, 51.72; H, 3.47; N, 8.04; S, 9.21. Found: C, 51.75; H, 3.48; N, 8.02; S, 9.23.

1-(2,5-dichlorophenylsulfonyl)-7-hydroxy-3,4-dihydroquinolin-2(1H)-one, Vd

Yield = 76 %. Mp 120–124 °C, FTIR (nujol) cm^{-1}: 1651 (-N-C=O), 1190 (-SO_2-N), 1077 (Ar-OH), 1041 (Ar-1,4-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.43 (s, 3H, N-CH_3), 3.19 (d, 2H, HN-CH_2-), 3.95 (t, 4H, -O-CH_2-CH_2-O-), 5.04 (t, 1H, -O-CH-O-), 7.32-7.66 (m, Ar-H). MS: m/z 372 (M+1); Anal. Calcd. for $C_{15}H_{11}ClNO_4S$: C, 48.40; H, 2.98; N, 3.76; S, 8.61. Found: C, 48.42; H, 2.99; N, 3.78; S, 8.60.

1-(4-tert-butylphenylsulfonyl)-7-hydroxy-3,4-dihydroquinolin-2(1H)-one, Ve

Yield = 73.6 %. Mp 136–140 °C, FTIR (nujol) cm^{-1}: 1681 (-N-C=O), 1376 (-C(CH_3)$_3$), 1191 (-SO_2-N), 1080 (Ar-OH). ^{1}H-NMR (300 MHz, $CDCl_3$): 2.43 (s, 3H, N-CH_3), 3.19 (d, 2H, HN-CH_2-), 3.95 (t, 4H, -O-CH_2-CH_2-O-), 5.04 (t, 1H, -O-CH-O-), 7.32-7.66 (m, Ar-H). MS: m/z 359 (M+1); Anal. Calcd. for $C_{19}H_{21}NO_4S$: C, 63.49; H, 5.89; N, 3.90; S, 8.92. Found: C, 63.51; H, 5.90; N, 3.91; S, 8.94.

DNA BINDING EXPERIMENTS

Electronic absorption titration

The absorption spectra of the compound **IVa** and **Va** are given in Fig 1. The experiments involving interaction of the organic compound with calf thymus DNA were performed in Tris HCl buffer (5mM tris(hydroxyl methyl)aminomethane, tris, pH 7.2, 50 mM NaCl) solution. CT-DNA in buffer solution gave a ratio of absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) of *ca* 1.84:1, indicating that the calf thymus DNA was free from protein [12]. The concentration of CT DNA per nucleotide was measured by using its known extinction coefficient at 260 nm (6000 M^{-1} cm^{-1}). Absorption spectra were recorded after each successive addition of DNA and equilibration (approximately 10 min). From the observed data, the intrinsic binding constant, K_b was calculated by using the following equation [12].

$$[DNA]/ (\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/ K_b (\varepsilon_a - \varepsilon_f)$$

where ε_a, ε_f, ε_b are the apparent, free and bound compound extinction coefficients. A plot of [DNA]/ (ε_b- ε_f) versus [DNA] gave a slope of 1/ (ε_b- ε_f) and a '*y*' intercept equal to $1/K_b(\varepsilon_b - \varepsilon_f)$, where K_b is the ratio of the slope to the *y* intercept.

Viscosity measurement

Viscosity measurements were carried out using an Ubbelodhe viscometer at room temperature. Flow time was measured by hand with digital stopwatch, each sample was measured three times and the average flow time was calculated. The data were reported as $(\eta/\eta_o)^{1/3}$ versus the binding ratio [13], where η is the viscosity of DNA in the presence of the compound and η_o is the viscosity of DNA solution alone. Viscosity values were calculated from the observed flow time of DNA containing solution corrected for the flow time of the buffer alone.

DNA cleavage experiment

The gel electrophoresis experiments using supercoiled pUC19 DNA were carried out as reported previously [14-16]. DMF solutions of both the compounds (**IVa and Va**) were placed in clean Eppendorf tubes and 1 µg of pUC19 DNA was added. The contents were incubated for 30 min at 37 °C. The samples were electrophoresed at constant voltage (70 V) on a 1 % agarose gel in Tris-Boric acid-EDTA (TBE) buffer. The gel was stained with a 0.5 µg/mL ethidium bromide and visualized by UV light and photographed for analysis. The extent of cleavage was measured from the intensities of the bands using the Alpha Innotech Gel documentation gel system (Alpha Imager 2200). For mechanistic investigation, reactions were carried out by adding radical scavenging agents to supercoiled DNA prior to the addition of the complex before incubation.

Antimicrobial Activity

The *in vitro* antimicrobial activity of the synthesized compounds was carried out against three bacterial species viz., *Bacillus Subtilis, Escherichia coli and Staphylococcus aureus* and two fungal species namely *Aspergillus niger and Alternaria solani* by cup-plate method [17]. The microbial strains were obtained from department of Biotechnology, Manasagangotri, University of Mysore, Mysore, Karnataka, India. Chloramphenicol and fluconazole were used as standard antibacterial and antifungal drugs, respectively. The solution of the synthesized compounds were prepared in DMSO and tested. The Minimum Inhibitory Concentration (MIC) study was carried out at different concentrations such as 100, 200, 300, 400, 500 and 1000 ppm. Since the zone of inhibition between 400 and 500 ppm were similar so 400 ppm was selected as MIC.

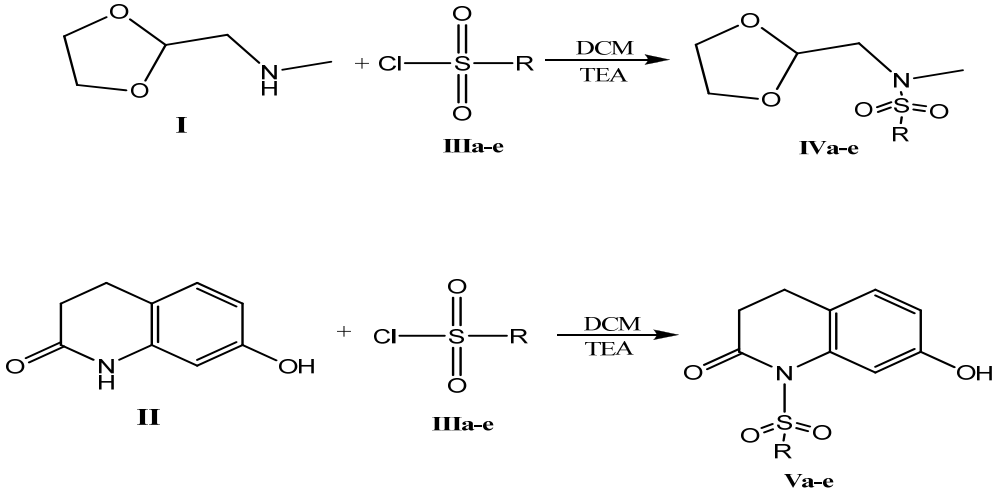
A test tube containing sterile melted soft agar (2 % in distilled H_2O, 6.0 mL) was maintained at 50 °C and inoculated with 0.2 mL suspension of the test culture, mixed thoroughly and poured in the Petri dish containing sterile agar medium and allowed to solidify for 10 min. the cup-borer was sterilized by dipping into absolute ethanol and flaming it and the allowed to cool down. The cups were marked in the agar with the help of sterile cup-borer and were injected with 100 µL of respective test sample solution of concentration 400 ppm in DMSO solvent, 100 µL standard drug (400 ppm) solutions in distilled water and 100 µL of DMSO as control, respectively. Then, the test sample was allowed to diffuse for 1 h in refrigerator at 4-5 °C. The plates were then incubated in upright position at 37 °C for 24 h for antibacterial and 72 h for antifungal activity respectively. The zone of inhibition surrounding each cup was observed and was expressed in percentage (Table 1).

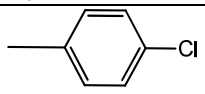
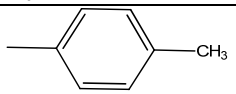
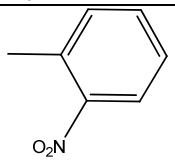
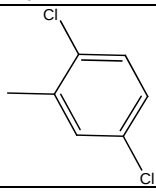
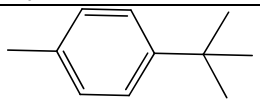
Anthelmintic Activity

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [18]. Six groups of six earthworms each were released in to 10 mL of solutions of piperazine citrate and test solution in DMSO. Piperazine citrate was used as reference standard while DMSO as control. The observations were recorded and tabulated in Table.2.

RESULTS AND DISCUSSIONS

N-((1,3-dioxolan-2-yl)methyl)-N-benzenesulfonamides (**IVa-e**) and 7-(4-bromobutoxy)-1-(phenylsulfonyl)-3,4-dihydroquinolin-2[1H]-ones (**Va-e**) were obtained by the reaction of 2-dimethylaminomethyl-1,3-dioxolane and 7-hydroxy-3,4-dihydroquinolin-2(1H)-one with various substituted sulfonyl chlorides. Examinations of analytical and spectral data of all the synthesized compounds are in good agreement with calculated values based on the proposed structure shown in Scheme I.



Compound	IIIa	IIIa	IIIa	IIIa	IIIa
R					

Scheme-I: Synthetic route of sulfonamides of 2-dimethylaminomethyl-1,3-dioxolane and 7-hydroxy-3,4-dihydroquinolin-2(1H)-one

DNA binding studies

Electronic absorption titration

The binding interaction of the CT-DNA with compounds **IVa** and **Va** was monitored by comparing their absorption spectra with and without CT-DNA. Both the compounds show minor bathochromic shift of the spectral band with significant hypochromicity, suggesting mainly groove binding propensity of the compounds to the DNA helix (Fig. 1).

This may be attributed to the presence of phenyl group that facilitates the interaction with double stranded DNA.

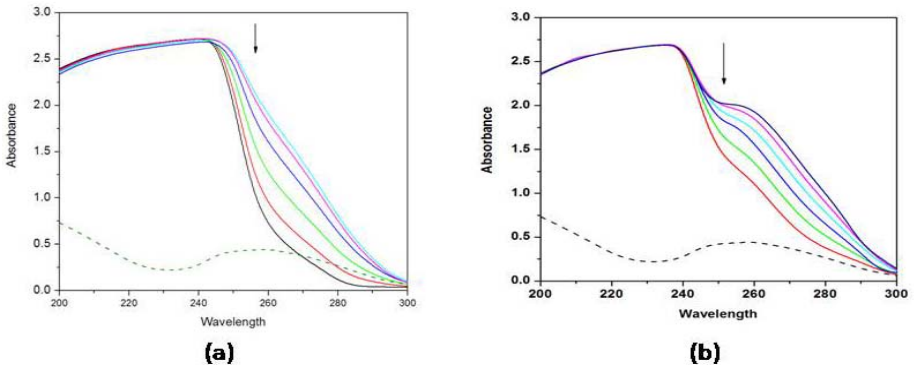


Fig. 1: Absorption spectra of (a) IVa and (b) Va in Tris-HCl buffer upon addition of DNA=0.5 µM, 0-50 µM. Arrow shows the absorbance changing upon increasing the concentration of test compound.

Viscosity measurement

To confirm the DNA binding modes, viscosity studies were carried out. A significant increase in the viscosity of DNA on addition of compound results due to the intercalation which leads to the separation among the DNA base pairs to the increase in the effective size in DNA which could be the reason for the increase in the viscosity [20]. Plot of $(\eta/\eta_o)^{1/3}$ versus [compound]/[DNA] gives a measure of the viscosity changes (Fig. 2). A gradual increase in the relative viscosity was observed on addition of the compound **IVa** and **Va** to DNA solution suggesting mainly groove binding nature of the compounds.

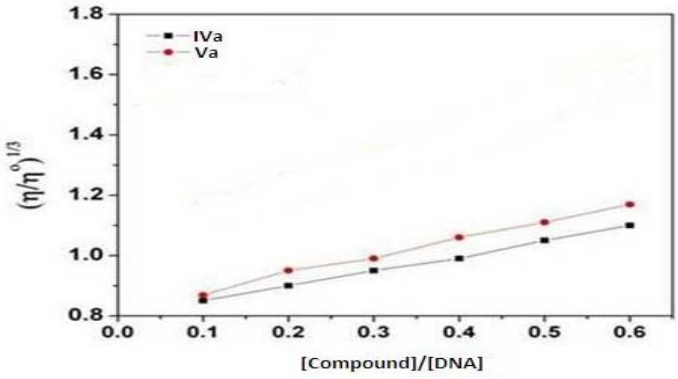


Fig. 2: Effect of increasing amounts of IVa (■) and Va (●) on the relative viscosities of CT-DNA at room temperature

Antimicrobial

The results of *in vitro* study of antimicrobial activity of newly synthesized compounds against three bacterial species (*Bacillus Subtilis, Escherichia coli and Staphylococcus aureus*) and two fungal species (*Aspergillus niger and Alternaria solani*) are reported in Table 1. Compounds **IVd** and **Vd** have shown excellent antibacterial activity against *B.Subtilis* and *E.coli* comparable to that of the standard, Chloramphenicol. They inhibited bacterial growth up to 84-91 % at 400 ppm concentration whereas compounds **IVa, IVc, Va, Vc** and **Ve** were found to have moderate antibacterial activity in the range of 50-77 % at 400 ppm concentration and the remaining compounds have poor antibacterial activity. Analogously, all the compounds exerted moderate to poor antifungal activity against *A.niger and A.solani* which were compared with standard drug (fluconazole).

Table 1: Results of antimicrobial activity on some selected bacteria and fungi (in percentage inhibition)

Compound	Antimicrobial activity (zone of inhibition in percentage)*				
	B. Subtilis	***E. coli***	***S. aureus***	***A niger***	***A. solani***
IVa	69.5	74.5	51.2	42.6	46.1
IVb	32.1	43.4	30.6	30.1	32.7
IVc	71.3	77.2	65.3	57.3	49.8
IVd	84.7	91.3	79.7	69.2	68.5
IVe	39.4	46.6	41.2	33.7	35.3
Va	73.6	79.7	61.3	62.4	53.6
Vb	41.1	41.9	32.1	30.2	36.1
Vc	77.0	73.1	67.5	56.8	59.9
Vd	89.5	87.3	76.9	65.7	66.2
Ve	62.6	63.7	58.3	43.9	48.3
Chloramphenicol	100	100	100	-	-
Fluconazole	-	-	-	100	100

*Average of three replicates

Anthelmintic activity

In this study, few of the compounds exhibited marked anthelmintic activity in terms of causing paralysis and death of worms (Table 2). The results revealed that compounds **IVc, IVd, Va** and **Vd** showed potent anthelmintic activity and rest of the compounds have shown better to poorer activity. The results were compared with standard drug piperazine citrate at same concentration.

Table 2: Results of Anthelmintic activity

Compound	Concentration (mg mL^{-1})	Time taken for paralysis (min)	Time taken for death (min)
Blank (Normal saline)	5	No effect till ten hours	No effect till ten hours
Standard (Piperazine citrate)	5	10	15
IVa	5	22	37
IVb	5	29	43
IVc	5	19	24
IVd	5	17	21
IVe	5	33	49
Va	5	17	20
Vb	5	31	38
Vc	5	21	28
Vd	5	16	19
Ve	5	29	36

DNA cleavage

The cleavage efficiency of the synthesized molecules **IVa** and **Va** was probed using agarose gel electrophoresis [21, 22]. Figure 3 shows the gel electrophoretic separations of plasmid pUC19 DNA induced by the compounds **IVa** and **Va**. The compounds were able to convert super coiled DNA (Form I) into open circular DNA (Form II) and/or linear DNA (Form III). Both the compounds showed effective cleavage in the presence of H_2O_2 (25 µM) as an oxidizing agent.

In the present study, the gel electrophoresis experiment was conducted at 35 °C using synthesized compounds **IVa** and **Va** in the presence and absence of H_2O_2 as an oxidant. It was found that, both the compounds exhibit nuclease activity in the presence and absence of H_2O_2. It was also noticed that, compound **Va** was effective towards cleavage than **IVa** and this may be due to the presence of hydroxyl group in **Va** which promotes better cleavage.

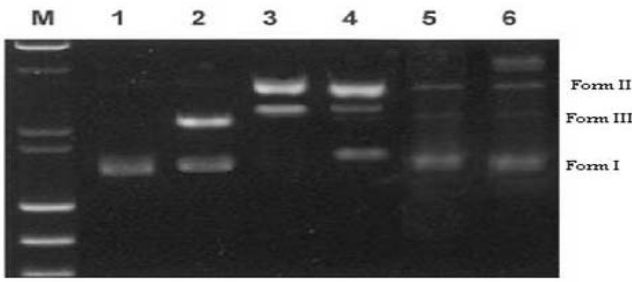


Fig. 3: Cleavage of supercoiled pUC19 DNA (0.5 µg) by the compounds IVa and Va in a buffer containing 50 mM Tris-HCl at 37 ^{0}C (30 min): lane M: marker; lane 1:DNA control; lane 2:DNA+H_2O_2; lane 3:IVa (10^{-3} M)+DNA; lane 4:IVa+DNA + H_2O_2; lane 5:Va (10^{-3} M)+DNA; lane 6:Va+DNA+H_2O_2.

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