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Antimicrobial properties and DNA interactions studies of 3-hetarylazoquinoline-2,4-diol compounds

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The purpose of the present study was to evaluate the antimicrobial effects of 3-hetarylazoquinoline-2,4-diols (**19**), having substituted or unsubstituted thiazole, benzothiazole, benzimidazole, triazole, isoxazole, and thiadiazole moieties at the 3-position, on bacterial and yeast strains. In addition, compounds and DNA interaction with pUC18 plasmid DNA were studied. The biological effects of these molecules were compared with potential antibiotics ampicillin, chloramfenicol (for bacteria), and ketoconozole (for yeast). According to the results, all compounds except **8** have mild to moderate activity against some of the bacterial strains tested, and none of the compounds have activity against the fungi *C. tropicalis* and *C. albicans.* Compounds **1** and **6** have moderate activities against *B. cereus* and *B. subtilis*, and compounds **5** and **6** have moderate activities and **1** has mild activity against *E. coli.* Compound **2** has a mild effect on all bacterial strains except *E. coli* ATCC 25922, *P. vulgaris*, and *S. aureus.* Compounds **3**-**5** and **7** have mild activity against some of the bacterial strains tested. Compound **9** has mild activity against all bacterial strains except *E. coli* ATCC 25922. Compound **8** has no antibacterial effect.

Compounds and DNA interaction results indicate that as the compounds bind to DNA, unwinding of supercoiled form I DNA takes place to change it from the negatively supercoiled form I to the nicked form II and linear form III DNA. The following studies showed that, among the synthesized compounds, **3**-**9** seem to be promising candidates possessing DNA cleavage activities besides anti-microbial properties to serve as chemical nucleases and chemotherapeutic agents.

Key Words: Quinoline-2,4-diol, azo dye, antimicrobial property, DNA interaction

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Introduction

Although many antimicrobial compounds are available, microorganisms' resistance to these drugs constantly emerges.[1] In the early 1970s, it was thought that virtually any microbial infection could be treated, as a wide range of antimicrobial agents (antibiotics) were available. That idea proved short-lived, when pathogens resistant to the conventional antibiotics routinely used to treat microbial infections emerged.[2] In order to prevent this critical medical problem, the production of new types of antibacterial agents is a very actual task[3] Furthermore, some dyes are used as drugs or in photodynamic therapy. The use of dye in therapy is now gaining importance against drug-resistant microbes. Among those, prontosil rubrum, an azo dye, is known to be a very effective pro-drug, and is widely used against bacterial infections[4,5] Quinoline compounds are most commonly, used as a main compound to make drugs (especially anti-malarial medicines), fungicides, and biocide agents. A large diversity of quinoline derivatives have been used as antimalarial, anti-inflammatory, anticancer, antibiotic, antihypertensive, and anti-HIV agents.[6] Several studies have been conducted to obtain new chemotherapeutic agents from quinolines. Further studies on the quinoline derivatives should be realized with regard to the safety issues regarding humans, formulations for improving the antimicrobial stability and effect, and antimicrobial mode of action.[7]

Although quinolines are very successful drugs, they are a poorly understood class of drugs. The quinoline drugs are similar to purine nucleotides; therefore proteins that interact with purines might also interact with quinoline.[8] Purine and pirimidines are subunits of DNA.

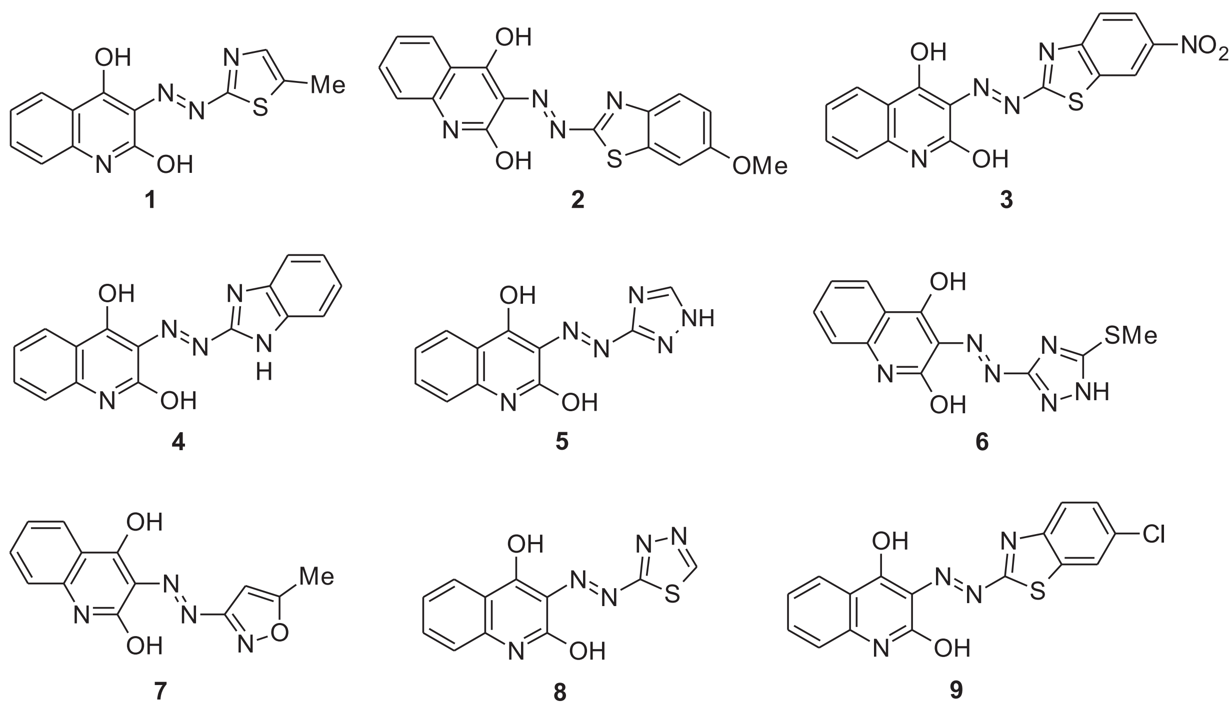
DNA is a very important biological molecule. Some parts of the DNA called genes code for the proteins. Genes regulate all the biochemical reactions on the cell via transcription and translation. DNA replication and gene expression are responsible for cell survival. For this reason, to destroy antitumor action, the drug is especially designed to bind DNA. Researchers are focusing on solving drug-DNA interactions. In order to solve this problem, they studied DNA replication origin and transcription, which are used as targets for a range of anticancer and antibiotic drugs. Drug binding causes structural and conformational changes in the DNA such as DNA bending, winding double or single strand breaks, resulting in DNA damage, which inhibits DNA transcription and replication.[9] In order to treat diseases drugs are designed to target DNA. Drug binding to DNA can be visualized in an electric field.[10] Quinolones are inhibitors of DNA gyrase and topoisomerase IV and cause bacterial cell death.[11] There is currently interest in studies about azo dyes having biogical activity.[12,13] There have been no reports in the literature so far about the activity against bacteria and DNA interactions with 3-hetarylazoquinoline-2,4-diol derivatives. In this study, we present nine 3-hetarylazoquinolin-2,4-dioles' activity in vitro against 8 clinical pathogen bacteria and 2 fungi, hoping that they might exhibit biological activity and be useful in the design of new biologically active azoquinolines. In addition, to gain further insight into their biological activities, the nature of plasmid DNA-interaction and inhibition of *BamH*I/*Hind*III restriction enzyme activity of the compounds were also studied for the first time.

Experimental

Synthesis of the compounds

3-Hetarylazoquinoline-2,4-diol compounds were synthesized according to the reported procedure;[14] hetarylamines (namely, 5-methylthiazol-2-amine, 6-methoxybenzothiazol-2-amine, 6-nitrobenzothiazol-2-amine, 1*H*-

benzimidazol-2-amine, 1*H*-1,2,4-triazol-3-amine, 5-methylisoxazol-3-amine, 1,3,4-thiadiazol-2-amine, and 6-chlorobenzothiazol-2-amine) were diazotized with nitrosylsulfuric acid, and then coupled with quinoline-2,4-diol in basic medium. The products (**1-9**) were insoluble in water, soluble in dimethyl sulfoxide (DMSO) and dimethylformamide, and slightly soluble in ethanol and methanol. The structures and the names of compounds are depicted in the Scheme and Table 1, respectively.



Scheme. The structures of azoquinoline-2,4-diols.

Table 1. Name of 3-hetarylazoquinoline-2, 4-diol compounds.

Compound no.	Compound name
1	3-(5-methylthiazol-2-ylazo)quinoline-2,4-diol
2	3-(6-methoxybenzothiazol-2-ylazo)quinoline-2,4-diol
3	3-(6-nitrobenzothiazol-2-ylazo)quinoline-2,4-diol
4	3-(1H-benzimidazol-2-ylazo)quinoline-2,4-diol
5	3-(1H-1,2,4-triazol-3-ylazo)quinoline-2,4-diol
6	3-(5-methylthio-1H-1,2,4-triazol-3-ylazo)quinoline-2,4-diol
7	3-(5-methylisoxazol-3-ylazo)quinoline-2,4-diol
8	3-(1,3,4-thiadiazol-2-ylazo)quinoline-2,4-diol
9	3-(6-chlorobenzothiazol-2-ylazo)quinoline-2,4-diol

Biological activities

The organisms employed in the antimicrobial screening system included 8 bacteria (*Staphylococcus aureus* ATCC 25923 (G+), *Pseudomonas aeruginosa* ATCC 27853 (G-) , *Escherichia coli* ATCC 35218 (G-), *Escherichia coli* ATCC 25922 (G-), *Bacillus subtilis* ATCC 29213 (G+), *Bacillus cereus* NRLL B-3008 (G+), *Proteus vulgaris* ATCC 8427 (G-), and *Enterobacter faecalis* ATCC 292112 (G+), and 2 fungi (*Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 13803). The bacterial strains were grown in nutrient agar medium and incubated at 37 °C for 24 h. The yeast cells were cultured on Sabouraud dextrose agar medium and incubated at 27 °C for 72 h. Chloramphenicol (30 μg) and ampicillin (10 μg) were used as standard antibacterial agents. All the experiments were performed 3 times, and the mean values are presented. Solutions of the compounds (5 mM) were prepared in DMSO. The compounds' effects were determined by the agar diffusion method.[15] Cultures were grown in exponential phase in nutrient broth at 37 °C for 18 h and adjusted to a final concentration of 10^8 cfu/mL by diluting fresh cultures and comparing to McFarland density. Medium was prepared, mixed with culture suspension, and poured into plates. Wells with a 6.0 mm diameter were made. Then 50 μL of solution of the test compound (0.25 μmol) was added to the well. After incubation, the diameter of inhibition zone was measured in millimeters.

Compound-DNA interaction

Interactions between compounds (**1-9**) and pUC18 plasmid DNA were studied by agarose gel electrophoresis. The compounds in DMSO were prepared and used freshly. Then 40 μL aliquots of decreasing concentrations of compounds ranging from 5000 to 156 μM were added to 1 μL of plasmid DNA (conc: 0.5 μg/mL) in a buffer solution containing TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4). The mixtures were left in an incubator at 37 °C for 24 h in the dark. Then 10 μL aliquots of compound/DNA mixtures were loaded onto the 1% agarose gel with loading buffer (0.1% bromophenol blue, 0.1% xylene cyanol). Electrophoresis was carried out in TAE buffer (0.05 M Tris base, 0.05 M glacial acetic acid, 1 mM EDTA, pH 8.0) for 3 h at 60 V.[16,17] After electrophoresis, the gel was subsequently stained by ethidium bromide (0.5 μg/mL) and visualized under UV light using a transilluminator (BioDoc Analyzer, Biometra) and photographed with a video camera and saved as a TIFF file. The experiments were performed 3 times.

*Bam*HI and *Hind*III restriction enzyme digestion

Compound-DNA mixtures were incubated for 24 h and then restricted with digestion by enzymes *BamH*I or *Hind*III (1 Unit) for 1 h at 37 °C. The restricted DNA was run in 1% agarose gel electrophoresis for 2 h at 60 V in TAE buffer.[18] The gel was stained with ethidium bromide and the gels were viewed with a transilluminator and the image captured by a video camera as a TIFF file.

Results and discussion

Compounds **(1-9)** were screened in vitro for their antibacterial activity against gram (+), gram (–), and fungal pathogens as described in the Experimental section. Table 2 summarizes the antibacterial and antifungal results. Most of the compounds display mild to moderate activities against *B. subtilis and B. cereus.* None of the compounds show any antifungal activity.

Table 2. Antimicrobial activity of compounds **1-9** expressed as inhibition zones (mm).

Test microorganisms	Compounds (5 mM)									Positive control			Negative control
	1	2	3	4	5	6	7	8	9	Chlo	Amp	K	DMSO
E. coli ATCC 35218	12.83 ± 2.36	10.33 ± 0.58	-	-	17.67 ± 0.29	18.33 ± .02	-	-	10.3 ± 0.58	36 ± 0.00	40 ± 1.00	-	-
E. coli ATCC 25922	-	-	-	-	-	-	-	-	-	25.66 ± 0.58	16.66 ± 1.15	-	-
B. cereus NRRL-B 37	16.5 ± 3.5	11 ± 0.00	11 ± 0.00	11.3 ± 0.76	-	16.5 ± 2.5	-	-	13 ± 0.00	26.33 ± 1.15	26 ± 0.00	-	-
B. subtilis ATCC 6633	16.33 ± 1.61	13.5 ± 0.5	12.17 ± 0.76	-	17.17 ± 2.25	15.5 ± 0.5	10.67 ± 0.29	-	10.67 ± 0.58	33 ± 00	26.33 ± 1.15	-	-
P. vulgaris	-	-	-	-	-	13.83 ± 0.29	-	-	9.5 ± 0.5	35.66 ± 1.52	10.33 ± 0.58	-	-
P. aeroginosa ATCC 27853	-	13.17 ± 0.29	-	-	-	-	-	-	11.5 ± 0.5	-	-	-	-
E. faecalis ATCC 292112	-	11.5 ± 0.5	-	-	-	-	-	-	12 ± 2.00	23 ± 1.73	28.33 ± 1.52	-	-
S. aureus ATCC 25923	-	-	11.67 ± 0.58	-	-	-	-	-	11.5 ± 0.5	30.33 ± 0.58	34.66 ± 0.58	-	-
C. tropicalis	-	-	-	-	-	-	-	-	-	-	-	30 ± 0.00	-
C. albicans	-	-	-	-	-	-	-	-	-	-	-	29 ± 0.00	-

Values represent averages ± standard deviations for triplicate experiments. (Amp: Ampicillin, Chlo: Chloramphenicol, K: Ketoconazole)

Compounds **5** and **6** are more potent against *E. coli* (G-) than **1**, **2**, and **9**, which indicated that 1,2,4-triazole rings in **5** and **6** are more effective than thiazole and benzothiazole moieties. Compounds **5** and **6** have similar activities against *E. coli* (G-) and *B. subtilis* (G+); in addition **6** has also moderate activity against *B cereus* (G+) and mild activity against *P. vulgaris* (G-). These additional activities of **6** might be attributed to thiomethyl substituent in the triazole moiety. However, compound **8** has no activity due to 1,3,4-thiadiazole moiety that has 1 sulfur atom instead of 1 nitrogen atom of 3 nitrogen atoms in the triazole ring. Triazole moieties in **5** and **6** have a N-H bond and 3 nitrogen atoms; therefore they are more capable of forming intra- and intermolecular hydrogen bonds than the others.

Compound **1** is more potent than **2**, **3**, and **9**. Compound **1** has 5-methylthiazole moiety and **2**, **3**, and **9** have benzothiazole moieties substituted with methoxy, nitro, and chloro, respectively. It might be due to benzo fused thiazole moiety, which has more electron delocalization, and also **8**, containing a benzo fused imidazole ring, has no activity against bacterial strains. Compounds **2**, **3**, **4**, and **9** are less potent with respect to the others. The lower effectiveness of those might be due to benzo annulation of corresponding azole moieties.

Compound **3** has mild activity against 3 gram-positive bacterial strains, and **2** has mild activity against 5 gram positive and gram negative bacteria. Compound **9** has mild activity all tested gram positive and gram negative strains except *E. coli* 25922. Comparing the substituent effects of **2**, **3**, and **9**, the chloro substituted derivative **9** has a broader effect against bacterial strains than **2** and **3**. It might be supposed that the chloro substituent is effective in broadening the bacterial effect and nitro and methoxy groups decrease the activity.

In order to determine whether compounds **1-9** cause conformational changes on the DNA helix, we studied the compounds' capacity to unwind supercoiling of closed circular pUC18 plasmid DNA as estimated by an electrophoretic mobility check on agarose gels. When plasmid DNA is subjected to electrophoresis, covalently closed circular form I and nicked circular form II bands can be seen on the gel (Figure 1). The closed circular form I DNA migrates faster than form II DNA. If both strands are cleaved, a linear form III DNA will be generated that migrates between form I and form II.[19,20] Figure 1 shows gel electrophoretic separation of pUC18 DNA after incubation with different concentrations from 5000 to 156 μM of the compounds. Untreated pUC18 DNA was used as the control. When plasmid DNA interacted with compound **1** and **2** is subjected to electrophoresis, 2 forms of DNA (form I and form II) are observed. In Figure 1, it can be seen that the intensity and mobility of form I plasmid DNA decrease gradually with the increasing concentrations of compound **1.** In the lowest concentrations of **1**, some of the supercoiled form I is modified and therefore split into 2 bands (Figure 1, Line 7), one of which has slower mobility and is most probably structurally different from the reference form I. In case of compound **3**, the mobility of form I decreases with increasing concentrations of the compound. In Figure 1 it can also be seen that the intensity of form I and II plasmid DNA gradually decreases with decreasing concentrations of **3**. In the 3 low concentrations of **3**, the supercoiled form I is separated into 2 bands, whereas form II diminishes gradually with decreasing concentration of **3**. A similar interaction can be seen for **4**: first the mobility of form I decreases with decreasing concentration of the compound, then the intensity of form II decreases at the 3 low concentrations of the compound. In the case of **5** and **6**, they unwind the form I supercoiled plasmid DNA. Compounds **5** and **6** exhibit a significant DNA cleavage of form I DNA. Compound **6** unwinds the plasmid pUC18 DNA and reduces the number of supercoils. This decrease in supercoiling of plasmid DNA causes a decrease in the migration through the gel, and eventually supercoiled form I and relaxed form II DNA comigrate (Figure 1). The cleavage of form I is observed with increasing concentration of **7**;

form I is split at high concentrations of the compound. In the case of **8**, interesting differences are observed; compound **8** causes an obvious migration of some of the supercoiled DNA molecules to lower molecular weight, possibly due to the degradation of the initial DNA. Similar degradative effects are observed for compounds **7** and **9**; modification of form I DNA has occurred, and form I is split into 2 bands one of which is faster.

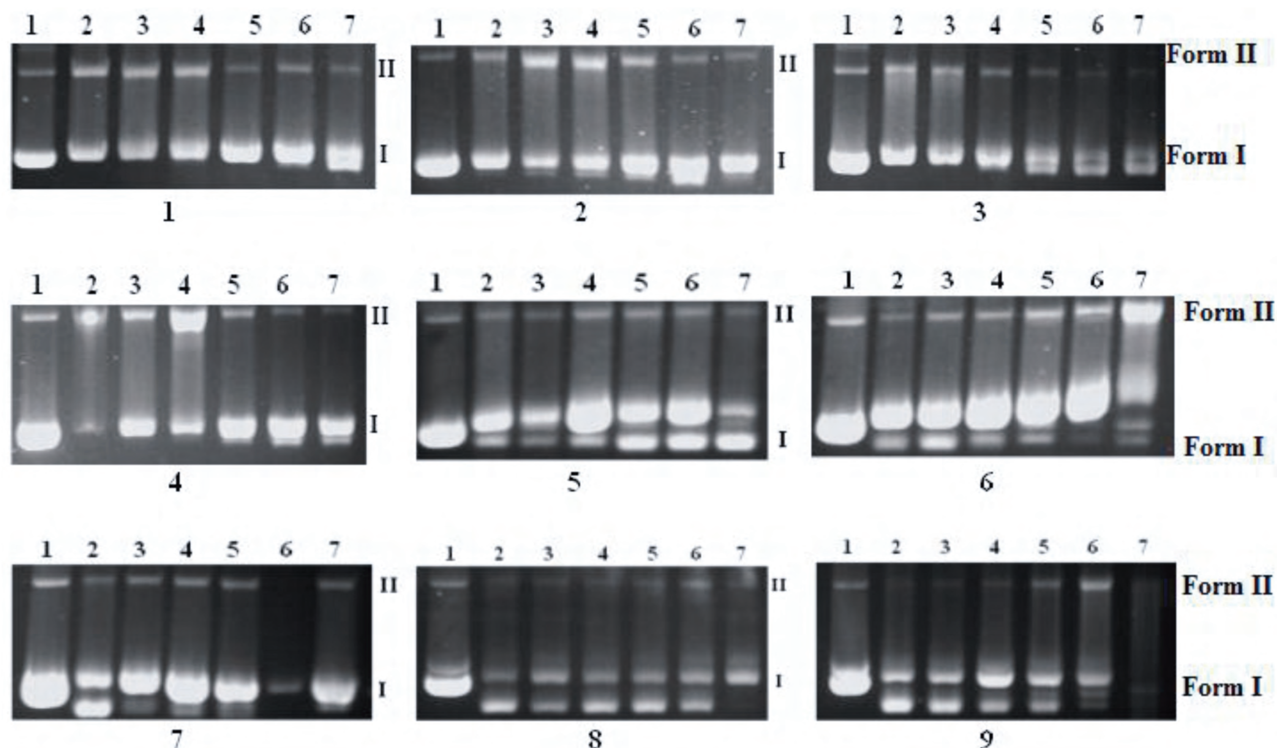


Figure 1. Modification of gel electrophoretic mobility of pUC18 plasmid DNA when incubated with various concentrations of compounds **1-9**. Concentrations (in μM) are as follows: (for compounds **1**-**9**: line 1 untreated pUC18 plasmid DNA; (line 2) 5000; (line 3) 2500; (line 4) 1250; (line 5) 625; (line 6) 312; (line 7) 156. The top and the bottom bands correspond to form II (single nicked open circular) and form I (covalently closed circular or supercoiled) plasmids, respectively.

Figure 2 shows the electrophoretograms applied to incubated mixtures of pUC18 plasmid DNA and compounds **1-9** followed by *BamH*1 digestion. After digestion of plasmid DNA with the enzymes, the linear form III band is not observed, indicating that the plasmid DNA/compound mixture is not restricted by the enzyme. The results suggest that compounds have affinity towards the GG region of the DNA.

Figure 3 shows the gel for the incubated mixtures of pUC18 plasmid DNA and compounds **1-9**, followed by *Hind*III digestion. In the case of *Hind*III digestion, 2 bands corresponding to form I and II were observed for all compounds. However, faint form III band was observed for compounds **3-9**. The results suggest that compounds **1** and **2** prevented digestion of enzyme, suggesting that the compounds bind to the AA region of the DNA. The other compounds (**3-9**) did not completely prevent restriction.

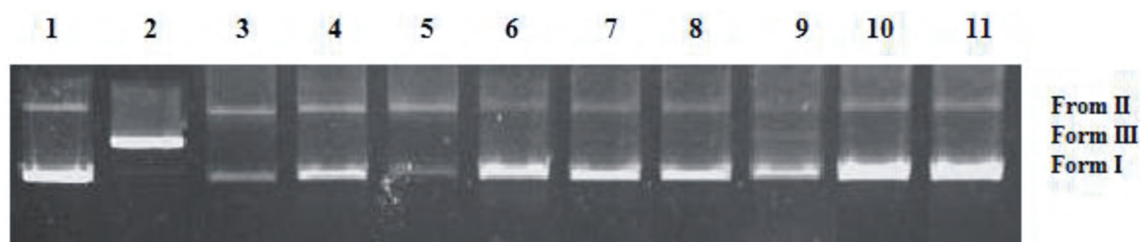


Figure 2. Electrophoretograms for the *Bam*HI digested mixtures of pUC18 plasmid DNA after their treatment with compounds **1-9**. (first and second lines) untreated pUC18 plasmid DNA and pUC18 DNA linearized by *Bam*HI, respectively. Line 3: compound **1**, line 4: compound 2, line 5: compound 3, line 6: compound 4, line 7: compound 5, line 8: compound 6, line 9: compound 7, line 10: compound 8, line 11: compound 9. Roman numerals I, II, and III indicate form I (covalently closed circular), form II (open circular), and form III (linear) plasmids, respectively.

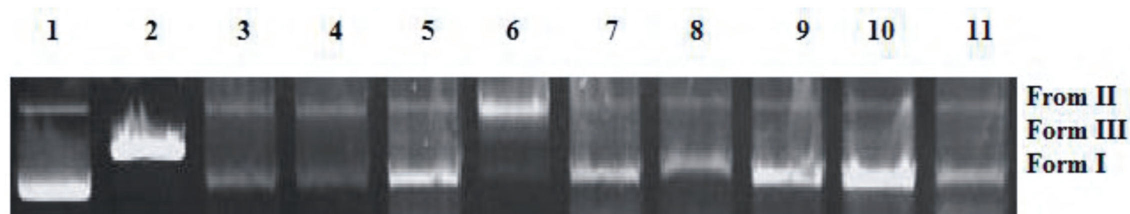


Figure 3. Electrophoretograms for the *Hind*III digested mixtures of pUC18 plasmid DNA after their treatment with compounds **1**-**9**. (Line 1) untreated pUC18 plasmid DNA and (and Line 2) pUC18 DNA linearized by *Hind*III. Line 3: compound **1**, line 4: compound **2**, line 5: compound **3**, line 6: compound **4**, line 7: compound **5**, line **8**: compound **6**, line 9: compound **7**, line 10: compound **8**, line 11: compound **9**. Roman numerals I, II, and III indicate form I (covalently closed circular), form II (open circular), and form III (linear) plasmids, respectively.

DNA is the most important cellular target for antibacterials and antitumor drugs.[21] Drugs can bind groves or intercalate into DNA. There are few reactive sites displayed on the surface of DNA sequences. Grove binders does not cause large conformational changes in DNA unlike intercalaters.[22] Intercalation into DNA results from insertion of compound between DNA base pairs, causing unwinding, stiffening, and lengthening of the DNA helix.[23] These modifications can promote functional changes, such as inhibition of replication, transcription, and DNA repair processes.[24] The structural modifications can be seen on agarose gel using plasmid DNA in 3 forms depending on conditions. Some drugs can twist and untwist or bend DNA depending on the kind of the DNA-compound interactions. Monofunctional intercalating adducts may untwist DNA, while bifunctional adducts form intra- and interstrand crosslinks and bend DNA.[17] Because the investigational drug design was focused on DNA, DNA interactions of 9 compounds were studied. Furthermore, plasmid DNA cleavage reaction was observed by agarose gel electrophoresis. The results show that all compounds tested have DNA binding activities, but compounds **5-9** have greater DNA binding activities than the other compounds, besides binding; in the case of **5-9** splitting of form I band was observed. The splitting bands may be indicating DNA cleavage. The presence of a small band on the gel for compound **5-9** may be also due to bending of DNA. In conclusion, it can be seen that all the compounds can bind and cleave plasmid DNA. Compounds **1-9** prevent *Bam*HI

enzyme digestion, suggesting that the compounds bind with GG nucleotides in DNA. Compounds 1 and 2 also prevent HindIII enzyme digestion, suggesting that 2 compounds have also affinity towards AA nucleotides in DNA. Binding modes are probably intercalative as all of the compounds are aromatic and also have hydrogen bond donors/acceptors. Although they are not very active against bacteria, they are quite active against double helix DNA. The antimicrobial activity of 9 compounds was tested against harmful bacteria. In summary, the compounds showed poor to moderate inhibitory activity against the both gram negative and mostly positive bacteria. The reason for that could be the protective outer membrane of gram negative bacteria. However, there was no activity against the tested fungi. Mechanism for quinolones can bind to the quinolone-resistance-determining region (QRDR) in the catalytic domain of the topoisomerase II or IV complex with DNA. Binding of compounds to DNA causes a variety of significant biological responses. DNA binding drugs inhibit DNA-protein interactions. Binding causes cell death by inhibiting the topoisomerase protein–DNA complex, thus interrupting normal DNA replication, resulting in oxidative damage and initiating cell-death mechanisms.[25] DNA gyrase is a specific target for quinolones in gram-negative bacteria, while topoisomerase IV is the main target in gram-positive bacteria.[26] Therefore, DNA targeted antibacterial agents offer an opportunity for the development of new antibacterial agents. The results obtained in this study could provide important knowledge for scientists working in this area.

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