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Research Note

Heteroalkyl oxime derivative can cause damage to bacterial DNA

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Abstract

The present study aimed to evaluate the antimicrobial activity of a hetroalkyl oximec synthetic compound derivative (Benzophenone O-4-bromoethyl oxime) against two gram positive and three gram negative bacteria. The antibacterial characteristic of this compound was shown using the circular zone diameter of bacterial growth inhibition by disk-diffusion method. In addition, the random amplified polymorphic DNA (RAPD) technique using twenty-three primers was performed to investigate the DNA polymorphism of bacteria that were affected by the synthetic chemical drug. Among these, only 18 primers were found efficient for reproducible banding pattern. Disappearance or appearance of bands between controls and treatments confirm rearrangements and DNA damages in the priming binding sites of bacterial genome. The results show that the RAPD method can be a useful identification tool for studying the DNA polymorphism created by synthetic chemical drugs. The results obtained with this technique showed significant differences between the RAPD profiles.

Keywords: Antibacterial; DNA Polymorphism; RAPD; synthetic drug

1. Introduction

In recent years, different imidazole and benzo imidazole drugs have been found to be associated with several biological activities such as antiparasitic, antifungal, anti-inflammatory, antibacterial activities [1-3]. Some imidazole and benozimidazole derivatives have surface activity and when used in high concentration are able to damage membranes. This situation is independent of the culture medium and growth rate. Imidazoles can be interacted directly with the lipid bilayer of the plasma membrane, probably by binding to the unsaturated fatty acids [4, 5].

Benzophenone O-4-bromoethyl oxime (Fig. 1) which is one of the members of the family of heteroalkyl oxime derivatives of imidizole and benzo imidazole, was designed and synthesized at the chemistry research laboratory. They may have a potent microbial activity against gram positive and gram negative bacteria. This paper presents the antimicrobial activity of these compounds and their damage to DNA.

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The disk-diffusion method is more suitable for routine testing in a laboratory where a large number of isolates are tested for susceptibility to numerous antibiotics. Growth of the organism and diffusion of the antibiotic commence simultaneously resulting in a circular zone of inhibition in which the amount of antibiotic exceeds inhibitory concentrations [6, 7].

Random amplified polymorphic DNA (RAPD) is used extensively for species classification and phylogenetic analysis [8-12]. A novel application of RAPD method is as a biomarker assay to detect DNA damage and mutational events, for example, rearrangements point mutation, small insert or deletions of DNA and ploidy changes in cells of bacterium, yeast, plant, and animal [13-19].

The aim of this study was to investigate the activity of a newly synthesized drug against gram-positive and gram-negative bacteria. Detection of bacterial DNA damage induced by benzophenone O-4-bromoethyl oxime, which was designed and synthesized at the chemistry laboratory, was carried out using the RAPD technique.

2. Materials and methods

2.1. Preparation of synthetic drug

Use of 1,4-dibromoethane and benzophenone oxime as substrates in the presence of K_2CO_3 as a base and anhydrous DMF at room temperature gave a satisfactory result and benzophenone O-4-bromoethyl oxime **(29b)** was obtained in good yield. Entry 1, benzophenone *O*-4-(1*H*-benzo[d]imidazol-1-yl)butyl oxime **(30e)** was synthesized in acetonitrile under reflux condition from the reaction between **(29b)** and benzimidazole in the presence of K_2CO_3 as the base. This product was isolated in 80% yield after 2 h (Fig. 1).

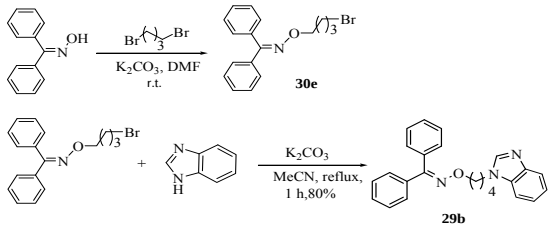

Fig. 1. Structure of synthesized drugs

2.2. Microbial culture

Five bacteria were taken from Persian Type Culture Collection (PTCC) of Iranian Research Organization for Science and Technology. There were three gram negative bacteria *Pseudomonas putida* (PTCC 1694), *Pseudomonas aeruginosa* (PTCC 1181) and *Xanthomonas campestris* (PTCC 1473) and two gram positive bacteria *Staphylococcus aureus* (PTCC 1431) and *Bacillus subtilis* (PTCC 1156). The bacteria (100 µL of each bacterial suspension with 0.5 McFarland concentrations) were grown in petri dishes which contained a layer of agar-based Müller-Hinton growth medium. Once the growth medium in the petri dish was inoculated with the desired bacteria, the plates were incubated 37 °C for 24 h [20].

2.3. Antibacterial disk-diffusion method

The antibiotic disks (6.4 mm of diameter) containing 10 µL of synthetic compound (50 µg/ml concentration) and or positive control antibiotics (Gentamicin against gram-negative and Penicillin against gram-positive bacteria) were placed on the agar plates streaked with bacterial suspension and inside a laminar airflow system. The antibiotic activity was measured using the circular zone diameter (mm) of bacterial growth inhibition by disk-diffusion method.

2.4. DNA extraction and RAPD experiment

Total genomic DNA of the five mentioned bacteria was extracted using GenElute Bacterial Genomic DNA kit (Sigma). The PCR amplification was carried out using twenty three 10-base pair random primers (Eurofins MWG Operon-company) with control and synthetic chemical drug treated bacterial genomic DNA as the template. PCRs were performed in a reaction mixture of 20 µl containing approximately 80 ng of the genomic DNA dissolved in sterile distilled water, 10X PCR buffer (2µl), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 2 µl of 10 µM primer and 1 U Taq DNA polymerase. The RAPD protocol consisted of an initial denaturing step of 5 min at 94°C, followed by 35 cycles at 94°C for 1min (denaturation), 37°C for 1 min (annealing), and 72°C for 2 min (extension), with an additional extension period of 10 min at 72°C. The PCR amplification products were separated on 1% agarose gel using Tris-Borate-EDTA (TBE) buffer and GeneRuler 100 bp DNA ladder (Fermentas, Germany). All the PCR examinations were carried out by Bioer XP thermal cycler and with two replications. Detection of genotoxic effect involves the comparison of RAPD profiles of DNA generated by control and treated bacteria.

3. Results

Table 1 shows the circular zone diameter (mm) of bacterial growth inhibition by disk-diffusion method for synthetic chemical drug, Gentamicin and Penicillin. The circular zone diameters of positive and negative controls were slightly higher than synthetic chemical drug. Table 2 shows the nucleotide sequences of the eighteen 10-mer primers which produced bands from twenty three examined primers by RAPD analysis. Table 3 is the summary of RAPD products obtained from five examined bacteria under control and synthetic chemical drug treated conditions. Total number of bands amplified from primers in each bacterium under normal and stress conditions was 35 to 123. In addition, average numbers of polymorphic bands per each primer were 0.7 to 4.7 percent. Figs. 2 to 5 are RAPD profiles of genomic DNA from five examined bacteria under control and synthetic chemical drug treatment.

Table 1. Circular zone diameter (mm) of bacterial* growth inhibition by disk-diffusion method

Sample	1	2	3	4	5
Synthesized compound	25	21/3	20	20/87	21/2
Gentamicin#	20/3	20/1	22/1	-	-
Penicillin$^{\Phi}$	-	-	-	22	21

*1- *P. putida* 2- *P. aeruginosa* 3- *X. compestris* 4- *S. aureus* 5- *B. subtilis*
#Gentamicin as positive control against gram-negative bacteria
$^{\Phi}$Penicillin as positive control against gram-positive bacteria

Table 2. Nucleotide sequences of the eighteen 10-mer primers* which produced bands by RAPD analysis

Primer	Nucleotide sequence (5´-3´)
1. OPA02	TGCCGAGCTG
2. OPA07	GAAACCGGTG
3. OPA08	GTGACGTAGG
4. OPA09	GGGTAACGCC
5. OPB07	GGTGACGCAG
6. OPB08	GTCCACACGG
7. OPD02	GGACCCAACC
8. OPD03	GTCGCCGTCA
9. OPD04	TCTGGTGAGG
10. OPD05	TGAGCGGACA
11. OPN01	CTCACGTTGG
12. OPN02	ACCAGGGGCA
13. OPN03	GGTACTCCCC
14. OPN04	GACCGACCCA
15. OPN06	GAGACGCACA
16. OPN08	CCTCCAGTGT
17. OPC14	TGCGTGCTTG
18. OPF14	GGTGCGCACT

*All primers were provided by Eurofins MWG Operon-Company (Ebersberg, Germany)

In this study, the gram negative bacteria *P. putida*, *P. aeruginosa* and *X. campestris* and the gram positive bacteria *S. aureus* and *B. subtilis* were used for investigation of synthetic chemical drug on bacterial DNA. *P. putida* is a rod-shaped saprotrophic soil bacterium [21]. *P. aeruginosa* is a common bacterium that can cause disease in animals, and human. It is found in soil, water, skin flora, and most man-made environments throughout the world [22]. *X. campestris* is a bacterial species that causes a variety of plant diseases [23]. *S. aureus* is a facultative anaerobic bacterium. It is frequently a part of the skin flora found in the nose and on skin, and in this manner about 20% of the human population are long-term carriers of *S. aureus* [24, 25]. *B. subtilis* is a rod-shaped bacterium commonly found in soil, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions [26].

4. Discussion

Table 3. Summary of RAPD products obtained from five examined bacteria* under control and benzophenone methyl oxime stress

Parameter	1		2		3		4		5	
	C	S	C	S	C	S	C	S	C	S
Total number of primers examined	23	23	23	23	23	23	23	23	23	23
Number of primers that produced bands	18	17	18	16	18	16	18	15	18	14
Total number of bands amplified from primers	86	91	123	78	120	59	117	64	106	35
Average number of bands per each primer	4.7	5.4	6.8	4.8	6.6	3.7	6.5	4.3	5.8	2.5
Total number of polymorphic bands	34.5	41	58.2	25	67.5	11	66	20	84.5	24
Average number of polymorphic bands per each primer	1.9	2.4	3.2	1.6	3.8	0.7	3.6	1.3	4.7	1.7
Polymorphic bands percentage from total bands	40.1	45	47.3	32	56.3	19	56.4	31.3	79.7	68.6

*1- *P. putida* 2- *P. aeruginosa* 3- *X. compestris* 4- *S. aureus* 5- *B. subtilis*

Antibacterial characteristic of synthetic chemical drug was studied using the circular zone diameter (mm) of bacterial growth inhibition by disk-diffusion method and by RAPD profiles of genomic DNA. The diameter of the inhibition zone is a function of the amount of drug in the disk and susceptibility of the microorganism. The circular zone diameters of bacterial growth inhibition in five examined bacteria were from 20 to 25 mm which confirm high inhibition of synthetic chemical drug on bacterial growth (Table 1).

Out of the 23 oligonucleotide primers tested, only 18 primers gave specific and stable results. As Table 3 shows, the number of primers that produced bands in each bacterium was higher in control compared to synthetic chemical drug treatment. This confirms that synthetic drug has changed the bacterial DNA. In addition, the comparison of RAPD profiles of genomic DNA from five bacteria between control and synthetic chemical drug treatment demonstrate obvious variations. Meanwhile, the primers gave a total of 879 bands ranging from 180-3000 base pairs in 10 gel electrophoresis (Figs. 2 to 6). Bacterial DNA damage was shown by RAPD profiles via absence or presence of bands. The total number of disappearing and appearing RAPD bands in profiles after heteroalkyl oxime treatment compared to the total control bands were 346 and 121, respectively. Disappearance of bands is likely due to changes in oligonucleotide priming sites, originated from rearrangements and DNA damage in the priming binding sites [19, 27-29]. Structural changes or variations in DNA sequences due to mutation and/or large deletions (bringing two pre-existing annealing sites closer) were created in new priming sites. Our finding and other reports support this claim that bacterial DNA polymorphisms detected by RAPD can be considered as a biomarker assay for detection of the genotoxic and DNA damage effects of natural or synthetic material with antibacterial property [30]. Previous studies had

shown that changes in DNA fingerprint offered a useful biomarker assay in toxicology [31, 19]. There is no a relationship between the circular zone diameters of bacterial growth inhibition in Table 1 and the DNA damage parameters in Table 3. It means that in the inhibition of bacterial growth some other mechanisms are also involved.

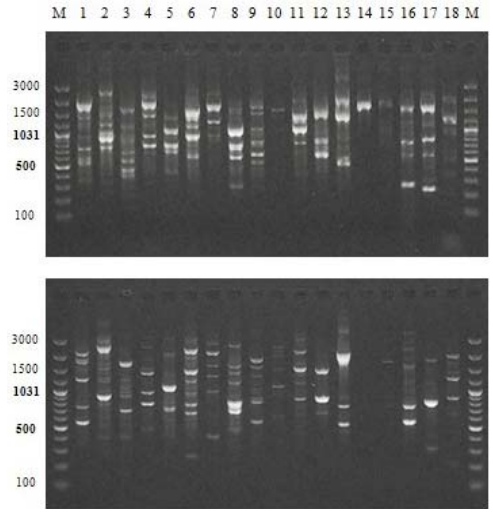

Fig. 2. RAPD banding profiles of genomic DNA from *P. putida*. Control (top) and benzophenone methyl oxime treatment (down). M: 100 bp DNA ladder (100-3000) and 1 to 18 are primers as shown in Table 1

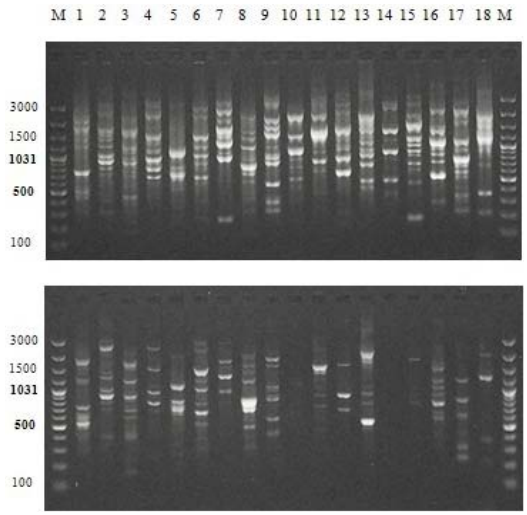

Fig. 3. RAPD banding profiles of genomic DNA from *P. aeruginosa*. Control (top) and benzophenone methyl oxime treatment (down). M: 100 bp DNA ladder (100-3000) and 1 to 18 are primers as shown in Table 1

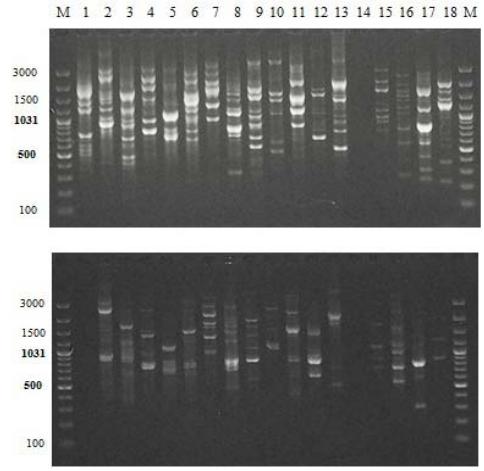

Fig. 4. RAPD banding profiles of genomic DNA from *X. compestris*. Control (top) and benzophenone methyl oxime treatment (down). M: 100 bp DNA ladder (100-3000) and 1 to 18 are primers as shown in Table 1

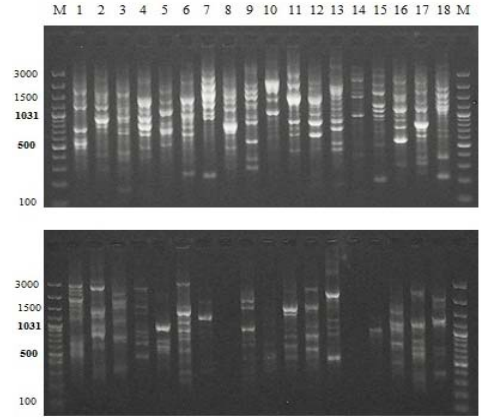

Fig. 5. RAPD banding profiles of genomic DNA from *S. aureus*. Control (top) and benzophenone methyl oxime treatment (down). M: 100 bp DNA ladder (100-3000) and 1 to 18 are primers as shown in Table 1

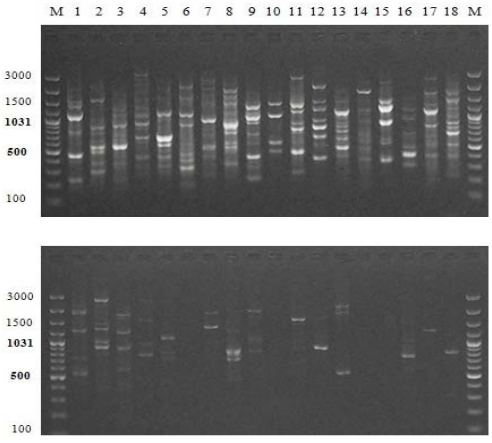

Fig. 6. RAPD banding profiles of genomic DNA from *B. subtilis*. Control (top) and benzophenone methyl oxime treatment (down). M: 100 bp DNA ladder (100-3000) and 1 to 18 are primers as shown in Table 1

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