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Original Research Article

Synthesis of novel polymers containing Schiff base as potential antimutagenic and antimicrobial agents

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ABSTRACT

In this research study, two novel polymers including Schiff base were prepared by condensation reaction of polystyrene-A-NH_2 with 5-nitro-2-thiophenecarboxaldehyde/or 5-(2-nitrophenyl)-2-furancarboxaldehyde. The synthesized polymers were evaluated using the elemental analysis, infrared spectroscopy (IR), gel permeation chromatography (GPC), thermal gravimetric analysis (TG/DTA). In addition, the antimicrobial and antimutagenic study of the polymers were reported. The antibacterial and antifungal activity were investigated using the diffusion method against pathogenic bacterial strains. PA-TC and PA-FC polymers exhibited the highest antimicrobial activity against the *S. typhi H.* and *P. vulgaris*, respectively. PA-FC polymer revealed the highest antifungal activity against *C. albicans*. The antimutagenic effects were evaluated using the sister chromatid exchange (SCE) and micronucleus (MN) methods against the sodium azide (NaN_3) in human lymphocyte cells. The results demonstrated that the PA-FC polymer have strong antimutagenic properties, especially in the concentration of 20 µg/mL.

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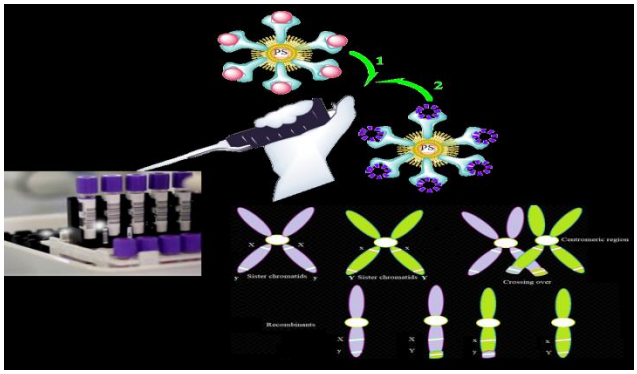
Graphical Abstract



Introduction

Polymeric Schiff bases containing azomethine linkage are of particular interest among polymers due to their remarkable properties such as mechanical, optical, magnetic, photoluminescence, catalytic, enzymatic, semiconducting, and electrochromic properties [1-5]. Polymeric Schiff bases and coordination polymers have high thermal stability, chemical resistance, scratch resistance, and corrosive resistance [6-8]. They also exhibit biological activities such as antimicrobial, anticancer, anti-inflammatory, antitumor, antifungal, antibacterial, antiviral, antimalarial, and antitumor activities [9-14]. In addition, the polymeric Schiff bases are used for the enzyme immobilization, for determination of pesticides and as electrochemical biosensor [15-18]. In summary, Schiff base polymers have potential applications in chemistry, biology, medicine, and pharmacetical industries [19, 20].

Mutagenicity is permanent changes that may cause hereditary alterations in the DNA of organisms [21]. Mutagens can cause some chronic diseases such as cancer, diabetes, rheumatoid arthritis, neurological, cardiovascular and hepatic disorders [22]. Antimutagens are substances that can prevent DNA damage caused by environmental factors from chemicals and toxic substances [23]. Sodium azide is one of the mutagenic chemicals. The mechanism of action of NaN_3 mutagenicity is the production of L-azidoadene, which interacts with DNA and causes mutation [24]. Antimutagenic agents can resist the effects of mutagens so it is important to investigate new compounds that may exhibit antimutagenic effect. Recently, examination on the antimutagenic properties of Schiff base polymers against NaN_3 were reported [25, 26].

The aim of this study was to evaluate the antimutagenic effects with the antibacterial and the antifungal activities of novel polymers including Schiff base. Condensation method was used for the synthesis. The well diffusion method, the sister chromatid exchange and micronucleus techniques were used to determine the antimicrabial and the antimutagenic properties. The biological activity was evaluated against the pathogenic bacteria. Antimutagenicity was determined against the sodium azide-induced mutagenicity.

Experimental

All chemicals were provided by the Sigma-Aldrich and Merck, and used without additional purification. Elemental analyses were carried out with a Leco CHNS-932 analyzer. IR were

recorded on a Perkin Elmer 100 FT-IR spectrometer at 4000-400 cm^{-1}. TG/DTA measurements were made in Perkin Elmer thermal analyser under a nitrogen atmosphere between 10 °C and 910 °C. GPC measurements were recorded using a Tosoh EcoSEC HLC-8320 GPC system.

Synthesis of polymers containing Schiff base ***(PA-TC)***

The polymer (PA-TC) was synthesized by condensation reaction (Figure 1a). Polystyrene-A-NH_2 (1 g, 0.8-1.2 mmol/g -NH_2 loaded) was dissolved in dimethylformamide (DMF) (20 mL). A solution of 5-nitro-2-thiophenecarboxaldehyde in DMF (10 mL) was then added dropwise to the boiling, stirred solution. The mixture was stirred and heated under a reflux condenser ca. 3.5 h, at 70 °C. The solution was cooled to room temperature and then purified with acetone. The purified polymer was filtered and dried in the oven for 24 h.

Synthesis of polymers containing Schiff base ***(PA-FC)***

The polymer (PA-FC) was synthesized by condensation reaction (Figure 1b). Polystyrene-A-NH_2 (1 g, 0.8-1.2 mmol/g -NH_2 loaded) was dissolved in DMF (20 mL). A solution of 5-(2-nitrophenyl)-2-furancarboxaldehyde in DMF (10 mL) was then added dropwise to the boiling, stirred solution. The mixture was stirred and heated under a reflux condenser ca. 3.5 h, at 70 °C. The solution was cooled to room temperature and then purified with acetone. The purified polymer was filtered and dried in the oven for 24 h.

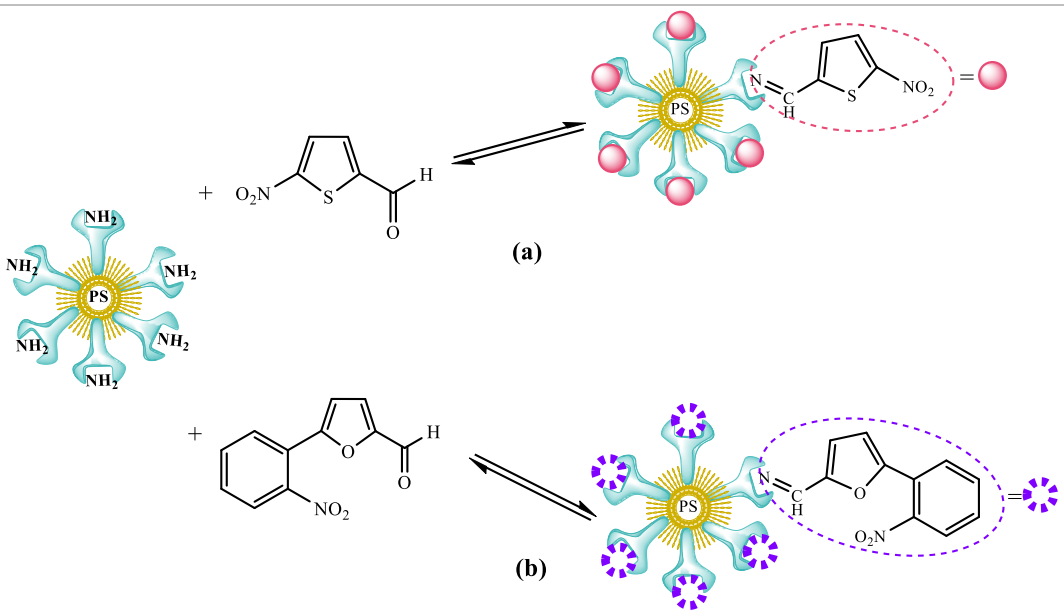


Figure 1. Synthesis of polymers containing Schiff Base **(PA-TC, PA-FC)**

Antimicrobial assay

The antibacterial and antifungal activity of the polymers containing Schiff Base (PA-TC, PA-FC) were examined against gram positive *Bacillus cereus* RSKK-863, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Listeria monocytogenes 4b* ATCC 19115, gram negative *Salmonella typhi H* NCTC901.8394,

Brucella abortus RSKK-03026, *Escherichia coli* ATCC 1280, *Klebsiella pneumonia* ATCC 27853, *Proteus vulgaris* RSKK96026 and the fungus *Candida albicans* Y-1200-NIH. This screening was performed in DMF solvent control using the well-diffusion method. The antimicrobial activity was not detected against any of the tested organisms. All the polymers were stored dry at room temperature and solved (3.5 μg/mL) in DMF 1% (v/v) of a 24-h broth culture including 10^6 CFU/mL was poured into sterile Petri dishes. The Molten Mueller Hinton agar was studied for culturing the test bacteria and and it was kept at 45 °C. The agar was poured into sterile petri dishes and was allowed for solidification. Then, holes of 6 mm diameter were pierced with sterile cork borer and the test solutions were added into each of the bores. Finally, the bacteria were incubated at 37 °C for 24 h. Average value determined for all the holes were used to compute the zone of inhibition growth [26].

Pathogenic bacterial cultures and yeast were evaluated for resistance to five antibiotics (produced by Oxoid Ltd., Basingstoke, UK): ampicillin, nystatin, kanamycin, sulphamethoxazole and amoxicillin. Ampicillin is a bactericidal that prevent the growth of gram (-) bacteria. Nystatin is an antibiotic that binds to sterols in the cell membrane of the fungus and changes membrane permeability. Kanamycin is a bactericide used in the treatment of infections sensitive to gram (-) and gram (+) microorganisms. Sulphamethoxazole is an antibacterial agent that affects the synthesis of folic acid in sensitive bacteria. Amoxicillin is a penicillin effective against microorganisms and it is an antibiotic used to treat bacterial infections [27].

Antimutagenic assay

The antimutagenic activities of the polymers containing Schiff Base (PA-TC, PA-FC) were investigated against NaN_3 in human lymphocyte cells by MN and SCE methods.

Peripheral blood lymphocytes were taken from four (two men and two women) healthy non-smoker donors. Lymphocyte cultures were composed as follows: RPMI 1640 chromosome medium+15% heat-inactivated fetal calf serum +1% streptomycin+1% penicillin +2% L-glutamine+2% phytohemagglutinin+0.5 mL of heparinized whole blood. NaN_3 (5 μM) was utilized as positive control.

The experiments were carried out as follows:
Culture 1: Solvent control;
Culture 2: NaN_3 (5 μM);
Culture 3: Polymers (80 μg/mL);
Culture 4: 5 μM NaN_3 + polymers (5 μg/mL);
Culture 5: 5 μM NaN_3 + polymers (10 μg/mL);
Culture 6: 5 μM NaN_3 + polymers (20 μg/mL);
Culture 7: 5 μM NaN_3 + polymers (40 μg/mL);
Culture 8: 5 μM NaN_3 + polymers (80 μg/mL);

In SCE assay, 5-bromo 2-deoxyuridine was inserted into the cultures at 6 mg/mL and incubated in darkness at 37 °C for 72 h. 0.1 mg/mL of colcemide was added to arrest mitosis at the metaphase stage. The cultures were centrifuged at 1200 g for 10 min and the supernatant used for enzyme analysis was discarded. For 25 min, cells were treated with a hypotonic solution (0.075 M KCl) and fixed in a 1:3 acetic acid/methanol (v/v) mixture. Metaphase chromosomes including bromodeoxyuridine were dyed with fluorescence plus Giemsa technique [28]. The results of SCE were recorded by selecting 60 metaphases. The well-spread second division metaphases including 42-46 chromosomes in each cell were scored for each treatment condition, and the values gained were computed as SCEs per cell [29].

In MN analysis, after 44 h of incubation, cytokalacin B (3 μg/mL) was inserted into the blood samples and incubated for 72 h. The cells were harvested after centrifugation, 6 mL of

0.05 M KCl was added and incubated for 7 min. After centrifugation again, 6 mL of fresh fixation solution was added to the cells. The cells were treated with 1 mL fixation solution and then dyed with 5% Giemsa stain. Slides were investigated with a microscope and 1000 bi-nucleated cells were scored [30].

Results and Discussion

Chemistry

Analytical data and physical properties of polymers containing Schiff base (PA-TC, PA-FC) are presented in Table 1. The values of weight average molecular weight (*Mw*), number average molecular weight (*Mn*) and polydispersity index (PDI) for polymers were obtained by the gel permeation chromatography [31]. Additionally, the values of the weight average molecular were determined by the elemental analysis.

Table 1. Thermal data and physical properties of polymers containing Schiff base

Compound	Chemical formula Colour, M_w[a]	(M_w, M_n), PDI	T_i (°C)	$T_{1/2}$(°C)	T_f (°C)	Residue mass at 900 °C (wt%)
PA-TC	$[(C_8H_8)_4(C_{15}H_{14}N_2O_2S)]$ Yellow, 702	(763, 647), 1,18	238,61	434,06	899,65	11,55
PA-FC	$[(C_8H_8)_5(C_{21}H_{18}N_2O_3)]$ Yellow, 866	(876, 595), 1,47	241,61	421,07	868,22	11,39

*Determined by elemental analyses

The characteristic infrared spectra of polymers containing Schiff base (PA-TC, PA-FC) are given in Table 2 and Figure 2. Imine bands for polymers were appeared in 1600 and 1602 cm^{-1}. This observation showed the addition of aldehyde to polymer containing amine group [32]. The IR bands, for polymers, in the 3015-3031, 2889-2892 and 1544-1546 cm^{-1} regions are characteristic aromatic ν(C-H), aliphatic ν(C-H) and ν(C=C) vibrations, respectively. The ν(CO) and δ(CO) vibrations of furan ring, for (PA-FC), were observed at 1502 cm^{-1} and 856 cm^{-1}, respectively. Also, ν(CSC) vibrations was appeared in 697 cm^{-1}, for (PA-TC).

Table 2. Infrared vibrations of polymers containing Schiff base

Compound	$\nu(CH)_{arom.}$	$\nu(CH)_{aliph.}$	ν(C=N)	$\nu(C=C)_{arom.}$	ν(CSC)	$\nu(CO)_{furan}$	$\delta(CO)_{furan}$
PA-TC	3015	2889	1600	1546	697	-	-
PA-FC	3031	2892	1602	1544	-	1502	856

*Determined by elemental analyses

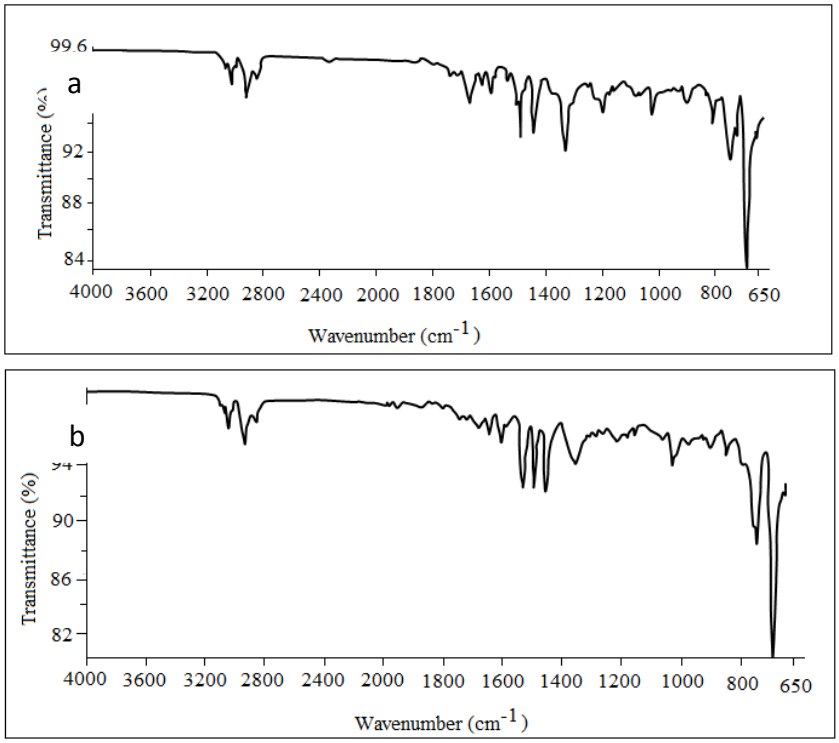


Figure 2. Infrared spectrums of polymers containing Schiff Base PA-TC (a), PA-FC (b)

Thermal analysis results of polymers containing Schiff base (PA-TC, PA-FC) are presented in Table 1 and Figure 3. Polymers (PA-TC, PA-FC) exhibited one-step weight. The values of initial (T_i) and finally (T_f) decomposition temperature were 239 °C and 900 °C, for PA-TC polymer. The values of (T_i) and (T_f) were 242 °C and 868 °C, for PA-FC polymer. According to the initial and finally decomposition temperature, the PA-TC is higher than that of the PA-TC. The high decomposition temperatures indicated the high thermal stability of the polymers (PA-TC, PA-FC). The TGA curve of PA-TC and PA-FC exhibited residue mass of 11, 55%, and 11, 39%, respectively. In the disintegration of the polymers containing Schiff base, these values corresponded to the percent of residual solid in polymer matrix at final temperature. It was observed that the temperature changes in the TGA curves of PA-TC and PA-FC polymers were over 100 °C. So the crystal water and solvent molecules were removed from polymers.

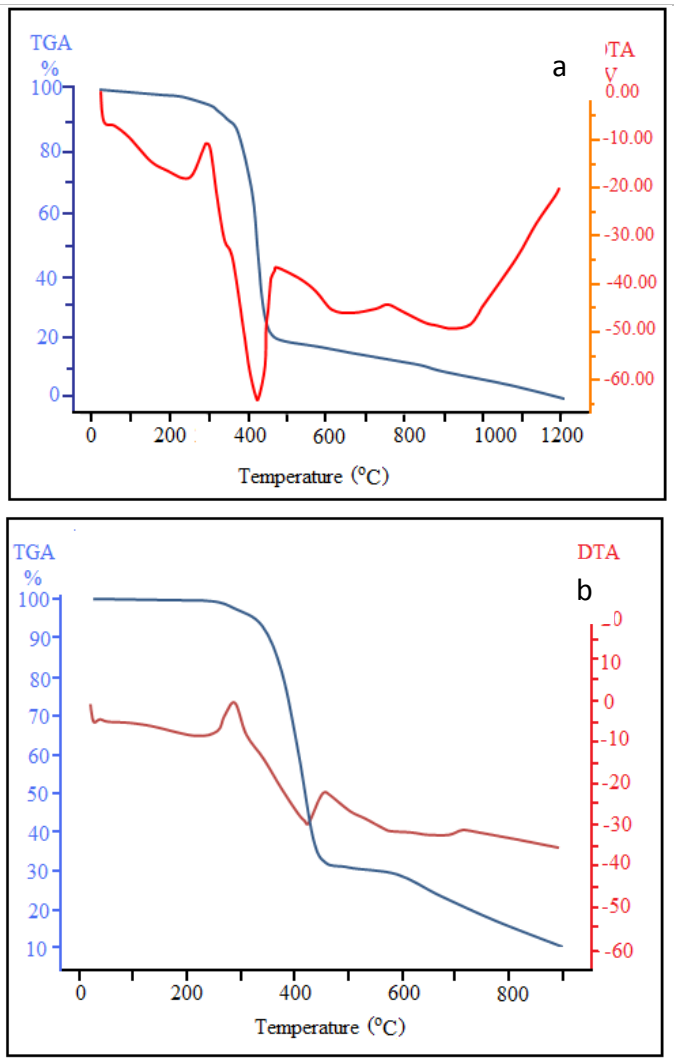


Figure 3. TGA/DTA curves of polymers containing Schiff base PA-TC (a), PA-FC (b)

Antimicrobial activity

The antimicrobial activities of polymers containing Schiff base (PA-TC, PA-FC) are presented in Table 3. The polymers were screened for antimicrobial activity against gram positive *B. cereus sp.*, *S. aureus*, *S. epidermis*, *M. luteus*, *L. monocytogenes 4b*, gram negative *S. typhi H.*, *B. abortus*, *E. coli*, *K. pneumonia*, *P. vulgaris* and the fungus *C. albicans* in DMF solvent control. The polymers and antibiotics were shown varying degree of inhibitory influences on the growth of varied selected pathogenic strains. PA-TC polymer was exhibited the highest antimicrobial activity against *S. typhi H.* The bacteria is caused diseases typhoid and paratyphoid fevers in humans [33]. PA-FC polymers was exhibited the highest antimicrobial activity against *P. vulgaris.* The bacteria is responsible for wound, burn, urinary tract and respiratory tract infections in humans [34]. Both polymers were showed high antifungal activity against the *C. albicans.* It is an opportunistic fungal pathogen and is caused bloodstream, mucosal and cardiovascular infections in humans [35]. Additionally, the antimicrobial activity of polymers was also check against five commercial antibiotics, so-

called Kanamycin (K30), Sulfamethoxazol (SXT25), Ampicillin (AMP10), Amoxycillin (AMC30) and Nystatin (NYS100). PA-TC and PA-FC polymers were showed higher antibacterial activity than K30 antibiotic, which is showed the highest activity for *S.typhi H.* Additionally, both polymer were showed higher antifungal activity than NTYS100 antibiotic. According to the obtained antibacterial and antifungal activitiy results, it can be conclueded that these polymers are pharmacologically active compounds.

Table 3. Antimicrobial activities of polymers containing Schiff base (diameter of zone of inhibition (mm))

Microorganisms	**Compound**		**Positive Control**				
	PA-TC	**PA-FC**	**K30**	**SXT25**	**AMP10**	**AMC30**	**NYS100**
S.aureus	-	13	25	24	30	30	-
P.vulgaris	16	25	--	--	--	--	--
L.monocytogenes 4b	-	20	--	--	--	--	--
E.coli	20	18	25	18	10	14	-
S.typhi H	30	21	20	17	11	19	-
S.epidermis sp.	20	16	--	--	--	--	--
Br. abortus	-	-	--	--	--	--	--
M.luteus sp.	12	-	--	--	--	--	--
B.cereus sp.	20	17	--	--	--	--	--
K.pneumonia	-	-	14	18	8	15	-
Candida albicans (Fungus)	27	32	-	-	-	-	20
DMF (solvent control)	-	-	-	-	-	-	-

Standart reagents: K30 Kanamycin, SXT25 Sulfamethoxazol, AMP10 Ampicillin, AMC30 Amoxycillin, NYS100 Nystatin, (--; (positive control) not tried)

Antimutagenic activity

The antimutagenic activities of polymers containing Schiff base (PA-TC, PA-FC) are presented in Table 4. The MN and SCE tests were used to assess the antimutagenic activities of different concentrations (5, 10, 20, and 40 µg/mL) of the polymers against NaN_3 in human lymphocyte cells. There is a relationship between the concentrations of these polymers and their protective roles. NaN_3 is a powerful, well-known mutagen that affects many organisms. It was identified that NaN_3 as causing DNA damage and the increase in MN and SCE frequencies determined in the control group was found as statistical significant ($p < 0,05$). A comparison was done between different concentrations of polymers added to the cultures to inhibit the genotoxicity caused by NaN_3. According to the obtained SCE and MN test system results, It was seen that PA-TC polymer increased the mutagenicity of NaN_3 by showing synergistic effect with NaN_3 used as positive control. It was determined that the PA-FC polymer decreased the mutagenic effect caused by NaN_3 by exhibiting antimutagenic properties against NaN_3, especially in the concentration of 20 µg/mL. It is known that the inhibitory activities of the compounds containing phenolic fragments are more effective [36]. It can be said that the antimutagenic effects of the compounds are associated to their action on the enzymatic activation system.

Table 4. The effects of polymers containing Schiff base and NaN_3

Groups	Concentrations	SCE/Cell ± S.E.	MN numbers ± S.E.
Solvent control		6.41 ± 0.24[a]	1.64 ± 0.12[a]
NaN_3	5 µM	10.20 ± 0.12[d]	3.42 ± 0.06[d]
PA-TC	80 µg/mL	7.24 ± 0.32[a]	2.32 ± 0.10[b]
NaN_3 + PA-TC	5 µM + 5 µg/mL	10.62 ± 0.14[e]	3.45 ± 0.06[d]
NaN_3 + PA-TC	5 µM + 10 µg/mL	10.71 ± 0.15[e]	3.62 ± 0.03[e]
NaN_3 + PA-TC	5 µM + 20 µg/ mL	10.78 ± 0.06[e]	3.74 ± 0.12[e]
NaN_3 + PA-TC	5 µM + 40 µg/mL	11.35 ± 0.16[e]	3.94 ± 0.09[e]
NaN_3 + PA-TC	5 µM + 80 µg/mL	11.48 ± 0.09[e]	4.02 ± 0.01[e]
PA-FC	80 µg/mL	6.84 ± 0.13[a]	2.06 ± 0.02[a]
NaN_3 + PA-FC	5 µM + 5 µg/mL	9.54 ± 0.05[d]	3.26 ± 0.09[d]
NaN_3 + PA-FC	5 µM + 10 µg/mL	9.02 ± 0.08[c]	3.02 ± 0.06[cd]
NaN_3 + PA-FC	5 µM + 20 µg/ mL	8.06 ± 0.019[b]	2.68 ± 0.04[bc]
NaN_3 + PA-FC	5 µM + 40 µg/mL	8.26 ± 0.17[b]	2.84 ± 0.01[c]
NaN_3 + PA-FC	5 µM + 80 µg/mL	8.74 ± 0.02[c]	2.98 ± 0.05[cd]

NaN_3 was used as positive controls for human lymphocytes.
PA-TC : $[(C_8H_8)_4(C_{15}H_{14}N_2O_2S)]$; PA-FC : $[(C_8H_8)_5(C_{21}H_{18}N_2O_3)]$.
[a, b, c, d, e] Statistically significant differences in the same column are indicated by the different superscripts (α=0.05).

Conclusions

In the present study, the antibacterial, antifungal and antimutagenic properties for the polymers containing Schiff base were investigated. Two novel polymers were synthesized using the condensation methods. The antimicrobial effects of these polymers were examined using the well-diffusion method against the selected pathogenic bacterial cultures and yeast. The results showed that these polymers containing Schiff base have strong / or moderate antimicrobial properties. The antimutagenic effects of the polymers against NaN_3 in human peripheral lymphocytes were investigated using the MN and SCE methods. PA-FC polymer, was shown to be the most effective in protecting the DNA from oxidative damage induced by NaN_3.

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Conflict of interest

We have no conflicts of interest to disclose.

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