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Study of the Effects on DNA of Two Novel Nucleoside Derivatives Synthesized as Potential Anti-HIV Agents

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The pursuit of antiviral active compounds against different classes of viruses, in particular HIV, HBV, and HTLV is an area of important and intense research. In the current study, two novel nucleoside derivatives belonging to a new class of isoxazolidine were successfully synthesized as potential anti-HIV agents by replacement of the furanose ring by a N,O-heterocyclic ring Both compounds were investigated for biological activity, namely, mutagenic and antimutagenic properties. Using *Salmonella typhimurium* strains TA97, TA100, and TA102, both compounds proved to be nonmutagenic, which may be considered an encouraging result to further elucidate other biological activities. Antimutagenic testing of the synthesized compounds revealed that they are active against the base-pair substitution mutagen sodium azide. However, they did not show any indication as antimutagenic agents against hydrogen peroxide and mitomycin C (oxidative mutagens) or against nitrophenylenediamine (a base-pair substitution and frameshift mutagen). Structure–activity relationship is also discussed. Testing these compounds as antiviral agents is highly recommended.

Keywords Antimutagenicity, 1,3-Dipolar cycloaddition, Isoxazolidine nucleosides.

INTRODUCTION

In the light of the absence of effective vaccines, the need of antiviral chemotherapeutic drugs is still emphasized for several respiratory tract infectious viruses (rhinovirus, adenivirus, parainfluenza, and the respiratory syncytial virus), herpesviruses, human papilloma viruses, in addition to most of the hemorrhagic fever viruses (DeClerq, 2002a).

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Modified nucleosides are of special interest because they are considered as one of the most important class of compounds potentially active against viral infection. Accordingly, the number of such derivatives is still growing exponentially year after year in response to the pressing need for new and effective antiviral agents. Such modified nucleosides have been proved to efficiently inhibit *in vitro* and *in vivo* virus infections caused by HIV, HBV, and HTLV-1 (DeClerq, 2002b, 2002c, 2004; Macchi et al., 2003). Similar mechanism of action has been proposed for many of the nucleoside analogues as inhibitors of viral replication. They act as reverse transcriptase inhibitor by serving as chain terminators after their intracellular phosphorylation to their 5′-triphosphate forms (Mitsuya et al., 1990; DeClerq, 1994; Katz and Skalka, 1994; Turner and Summers, 1999; Jonckheere et al., 2000). In this regard, a relatively new class of isoxazolidine modified nucleosides was synthesized by replacing the furanose ring by a N,O-heterocyclic ring (Chiacchio et al., 2003a, 2003b). Carboxylic 1′-oxa-2′-aza-nucleosides (Figure 1) are emerging as a new important class of compounds that are interesting to synthesize in order to investigate their biological activities (Borrello et al., 2003). In the current work, we aim at the synthesis of two novel isoxazolidine modified nucleoside derivatives as potential antiviral agents. On the other hand, studying the genotoxicity of these newly synthesized derivatives is of great importance to ensure their safety before proceeding toward their practical use. In addition, several investigators detected antimutagenic activities of different nucleoside derivatives (Sideropoulos, 1979; Ohta et al., 1986; Marquardt et al., 1988; Kopsidas and MacPhee, 1996; Yoshikawa et al., 2002). Furthermore, it is well-known that the great majority of carcinogenic compounds are also mutagenic (McCann and Ames, 1976; Rosenkranz and Poirier, 1979; Simmon, 1979). Lee and Lin (1988) investigated the antimutagenic activities of extracts of 36 commonly used anticancer crude drugs from Chinese herbs using the *Salmonella*/microsomal system in the presence of picrolonic acid or benzo[a]pyrene to test whether they contain direct or indirect antimutagens. The extracts of *Pteris multifida* P. showed the highest antimutagenic activity against picrolonic

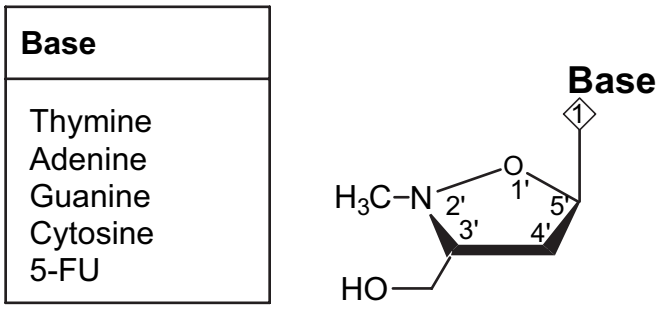


Figure 1: General structure of carboxylic 1′oxa-2′-azanucleosides.

acid–induced mutation. Moreover, several reports indicated that cancer is largely caused by damage to DNA (Hiatt et al., 1977; Schapper et al., 1982). Accordingly, the antimutagenicity of the investigated compounds could be a strong indication for their potential activity as anticancer agents.

MATERIALS AND METHODS

Chemicals

The test compounds, (3′SR,5′SR)-2′-methyl-3′-hydroxymethyl-1′,2′-isoxazolydinyl-α-adenine (**8b**) and (3′SR,5′SR)-2′-methyl-3′-hydroxymethyl-1′,2′-isoxazolydinyl-thymine (**7, 8a**) were synthesized and characterized as described under the Experimental section.

The structures of these compounds were confirmed by elemental analysis, IR, H^1 NMR, C^{13} NMR, and UV-spectral data.

NADP and glucose-6-phosphate were purchased from Boehringer Mannheim (Manheim, Germany). Mitomycin C (MMC) and phenobarbital were purchased from BDH Chemicals Ltd. (Poole, England), β-naphthoflavone was purchased from Aldrich Chemical Co. (Milweukee, WI, USA), 4-nitro-*o*-phenylenediamine (NPD) and dimethyl sulfoxide (DMSO) were obtained from Janssen Chimica (Beerse, Belgium), hydrogen peroxide (H_2O_2) was purchased from CBH Lab Chemicals (Nottingham, UK), D (+) biotin from Janssen Chimica (Beerse, Belgium), ampicillin from NenTech, Ltd. (UK), and L-histidine, tetracycline, and sodium azide (SA) from Sigma Chemical Company (St. Louis, MO, USA). For the preparation of bacterial growth media, the Oxoid nutrient broth no. 2 (Oxoid, Basingstoke, UK), the Difco Bacto nutrient broth (Difco, Detroit, MI, USA), and agar powder (Gainland Chemical Company, Deeside, UK) was used, as well as dextrose anhydrous extrapure (Laboratory Rasayan, S.D. Fine-Chem Ltd. Biosar SD's, Boisar, India). All other chemicals were of analytical grade.

Bacterial Strains

The *Salmonella typhimurium* strains TA97, TA100, and TA102 used in this study were kindly supplied by Prof. B. N. Ames (University of California, Department of Biochemistry). The bacterial strains were maintained on master plates as described by Maron and Ames (1983).

The *Salmonella* Mutagenicity Assay

The spot test (with and without S9 mix), the standard plate incorporation assay (without S9 mix), and the preincubation assay (with S9 mix) were carried out as described by Maron and Ames (1983). The rat-liver S9 fraction was prepared as described by Ono et al. (1994). Young male Sprague-Dawley rats weighing approximately 200 g were supplied by Yarmouk University Animal

House Unit (Jordan) and were used after induction with phenobarbital and β-naphthoflavone. The S9 mix (50 mL) contained 5 mL of induced rat liver S9; this corresponds with a protein concentration of approximately 40 mg/mL (Maron and Ames, 1983).

For the spot test, four concentrations of the tested chemicals were used, while for the standard plate incorporation assay and the preincubation assay at least 10 concentrations were used. All concentrations were spaced at about one-third interval. The highest concentration was restricted by the solubility of the tested nucleoside derivatives in DMSO. Triplicate plates were made for each dose, and each experiment was repeated independently at least twice in separate days.

The Antimutagenicity Assay

The antimutagenic activity of **7, 8a** and **8b** was analyzed using a modified preincubation assay (Gichner et al., 1994). The principle of the test was to incubate the tested compound with the authentic or diagnostic mutagen for 30 min at 37°C with shaking at 150 rpm. Then the bacterial strain was added and incubated for further 30 min. At the end of the incubation period, 0.5 mL was removed from this mixture and added to 2.5 mL of a molten top agar, which was incubated in a water bath at 45°C. The contents were mixed and then spread onto a minimal glucose agar plate. If any suspected antimutagen is added to a test tube containing a mutagenic compound, it could inhibit or decrease its mutagenic activity, given that it has antimutagenic activity. For each test chemical, six doses starting from 660 μg/plate were tested separated by about one-third interval or a multiple of it. In the current investigation, NaN_3 4 μg/plate, NPD 8 μg/plate, and H_2O_2 20 μL/plate were used with strains TA97, TA100, and TA102, respectively. The highest tested dose was selected to produce a significant reduction in the number of revertant colonies accompanied by no inhibition of bacterial background lawn growth. Triplicate plates were poured for each dose, and each experiment was repeated at least twice in separate days. In this test, three types of controls were made (negative, positive, and chemical). The negative control contained the buffer with the bacterial suspension only. The positive control contained the buffer, the bacterial suspension, and the mutagen. The chemical control contained the buffer, the bacterial suspension, and the tested chemical at its highest nontoxic concentration. The reduction in the number of the induced revertants is demonstrated by comparing the number of revertants on the antimutagen-containing plates with that on the antimutagen-free plates. The percentage of mutagenic repression was then calculated according to Gichner et al., (1994) using the following formula:

$$\% \text{ Mutagenic repression} = \left(1 - \frac{\text{experimental} - \text{negative control}}{\text{positive control} - \text{negative control}}\right) \times 100\%$$

Experimental

Chemistry

Melting points were determined on a Mettler FP80 (Toledo, OH) and are uncorrected. Elemental analysis was performed with a Perkin-Elmer elemental analyzer (Wellesley, MA). Infrared spectra were recorded on a Perkin-Elmer 377 instrument. ^{1}H and ^{13}C NMR spectra were measured on a Varian Gemini 300 (Paloalto, CA). Chemical shifts are reported in ppm (δ) from TMS as internal standard. NOE difference spectra were obtained by subtracting alternatively right-off resonance free induction decays (FIDS) from right-on resonance induced FIDS. Mass spectral data were obtained on a GC-MS HP 5995A (Sigma, St. Louis, MO). Silica gel 60–320 mesh was employed for routine column chromatography separation. Compounds were detected with a 254-nm UV lamp. Nitrones (**2**) and the thymidine modified nucleoside (**7, 8a**) were prepared according to a procedure already reported in literature (Tufariello, 1984).

Reaction of Nitrone 2 with Vinyl Acetate

Nitrone (3.6 mmol) was stirred with 25 mL of vinyl acetate in a sealed tube at 70°C, until the thin layer chromatography (tlc) showed the disappearance of the starting nitrone (24 h). The reaction mixture was evaporated under reduced pressure, and the residue was subjected to column chromatography on silica gel column with cyclohexane-ethyl acete 6:4 as eluent. Reaction of C-carboxyethyl-C-methyl-N-methylnitrone with vinyl acetate. First elution gave *trans* (3SR,5SR)-2,3-dimethyl-3-carboxyethyl-5-acetoxyisoxazolidine (**3**) 40% yield. Light-yellow color oil. ^{1}H NMR δ ($CDCl_3$): 1.27 (t, 3H), 1.41 (s, 3H, $H_{3'}$), 2.06 (s, 3H, CH_3CO), 2.19 (dd, 1H, H_{4a}), 2.65 (s, 3H, NCH_3), 3.17 (dd, 1H, H_{4b}), 4.18 (q, 2H), 6.26 (dd, 1H, H_5). ^{13}C NMR: δ ($CDCl_3$) 14.18, 19.37, 21.20, 38.99, 46.17, 61.79, 70.13, 95.20, 170.49, and 171.28. Further elution gave *cis* (3SR,5RS)-2,3-dimethyl-3-carboxyethyl-5-acetoxyisoxazolidine (**4**) 40% yield. Light-yellow color oil. ^{1}H NMR δ ($CDCl_3$): 1.28 (t, 3H), 1.35 (s, 3H, $H_{3'}$), 2.07 (s, 3H, CH_3CO), 2.56 (dd, 1H, H_{4a}), 2.73 (s, 3H, NCH_3), 2.95 (dd, 1H, H_{4b}), 4.21 (q, 2H), 6.23 (dd, 1H, H_5). ^{13}C NMR: δ ($CDCl_3$) 14.00, 16.84, 21.23, 38.6, 47.06, 61.66, 69.14, 94.38, 170.64, and 171.31.

*Reaction of Isoxazolidine (**3, 4**) with Silylated Adenine*

A suspension of adenine (23 mmol) in 30 mL of dry CH_2Cl_2 was treated with N,O-bis (trimethylsilyl) acetamide (75 mmol) and refluxed for 15 min; to the clear solution obtained was added a solution of isoxazolidine **3** and **4**, in dry CH_2Cl_2 (5 mL), and then trimethylsilyltriflate (5 mmol) was added; the reaction mixture was refluxed for 1 h. After being cooled to 0°C, the solution was neutralized by careful addition of aqueous 5% sodium bicarbonate and then it was concentrated in vacuum. To the crude was added dichloromethane

(30 mL), and the organic phase was separated, washed with water (2 × 20 mL), dried over sodium sulfate, filtered, and evaporated to dryness. The residue was purified by column chromatography (cyclohexane–ethyl acetate 3:2).

*Reaction of Isoxazolidine (**3, 4**) with Silylated Adenine*

The first fraction gave the *cis* (3′SR,5′ SR)-2′-methyl-3′-carboxyethyl-1′,2′-isoxazolydinyl-α-adenine **5b**, yield 40% as a reddish oil, ^{1}H NMR δ ($CDCl_3$): 1.31 (t, 3H, CH_3), 2.87 (s, 3H, NCH_3), 2.96 (dd, 1H, $H_{4a'}$), 3.26 (ddd, 1H, $H_{4b'}$), 3.46 (dd, 1H, $H_{3'}$), 4.24 (q, 2H), 5.96 (bs, 2H, NH_2), 6.51 (dd, 1H, $H_{5'}$), 8.33 (s, 1H, H_2), 8.44 (s, 1H, H_8). ^{13}C NMR δ ($CDCl_3$): 13.9, 29.45, 40.59, 44.41, 61.47, 68.69, 82.62, 119.53, 149.10, 152.73, 155.63, and 169.03.

The second fraction eluted is the *trans* (3′SR,5′ RS)-2′-methyl-3′-carboxyethyl-1′,2′-isoxazolydinyl-α-adenine **6b**, yield 20% as a brownish oil, ^{1}H NMR δ ($CDCl_3$): 1.25 (t, 3H, CH_3), 2.82 (s, 3H, NCH_3), 3.09 (m, 2H, $H_{4a' \text{ and } b'}$), 3.4 (dd, 1H, $H_{3a'}$), 4.22 (q, 2H), 6.34 (m, 1H, $H_{5'}$), 6.72 (bs, 2H, NH_2), 8.0 (s, 1H, H_2), 8.37 (s, 1H, H_8). ^{13}C NMR δ ($CDCl_3$): 13.99, 29.45, 44.41, 61.83, 69.96, 85.1, 118.64, 149.37, 149.37, 155.66, 154.9, and 169.03.

*Reaction of Nucleosides (**6b**) with $NaBH_4$*

To a stirred solution of **6b** (10 mmol) in a 1:1 methanol:dioxane mixture (100 mL) was added at 0°C $NaBH_4$ (50 mmol) and the obtained mixture was stirred for 5 h. at the end of this time, the solvent was extracted with ethyl acetate (3 × 30 mL), and the collected organic phase, dried over sodium sulfate, gave after evaporation of the solvent at reduced pressure a white solid, which was purified on column chromatography ($CHCl_3$–CH_3OH 97:3).

The first fraction gave (3′SR,5′ SR)-2′-methyl-3′-hydroxymethyl-1′,2′-isoxazolydinyl-α-adenine **8b**, yield 40% as white solid, ^{1}H NMR δ (DMSO-d_6): 2.56 (m, 1H, $H_{4a'}$), 2.72 (s, 3H, NCH_3), 3.01 (m, 2H, $H_{4'b}$), 3.53 (m, 2H, CH_2OH), 4.87 (dd, 1H, $H_{3'a}$), 6.20 (dd, 1H, $H_{5'}$), 7.07 (bs, 1H, OH), 7.27 (bs, 2H, NH_2), 8.15 (s, 1H, H_2) 8.36 (s, 1H, H_8). ^{13}C NMR δ (DMSO-d6): 36.73, 41.22, 59.78, 81.83, 108.82, 119.08, 149.20, 152.7, 156, and 146.04.

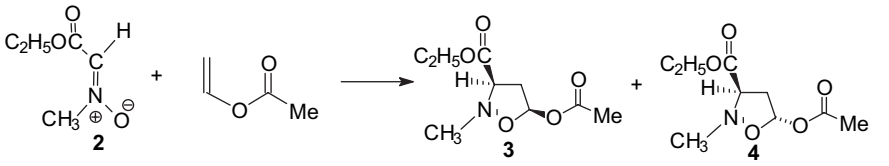
RESULTS AND DISCUSSION

Chemistry

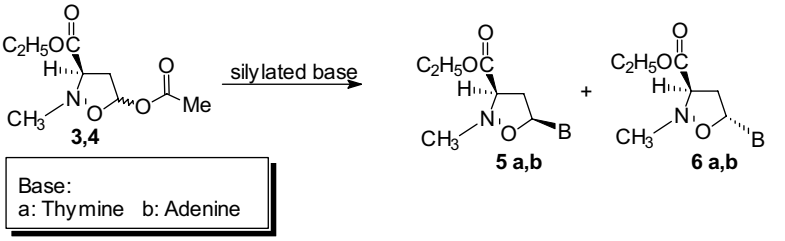
The synthetic scheme is based on pericyclic 1,3-dipolar cycloaddition (Chiacchio et al., 1993; Tufariello, 1984) of the C-ethoxycarbonyl-N-methyl nitrone **2** with vinylacetate (Chiacchio et al., 1996) followed by nucleosidation performed with silylated nitrogen base, and finally by the reduction of carboethoxy side chain into the 4′-hydroxymethyl 2′,3′dideoxynucleoside (Chiacchio et al., 1996). Nitrone **2** was reacted with vinylacetate in the absence of solvent, using 1:10 ratio of dipole to dipolarophile at room temperature, until

tlc showed the disappearance of the spot of the starting nitrone producing epimeric isoxazolidines **3** and **4** in a 1:1 relative ratio (80% combined yields) as shown in Scheme 1.

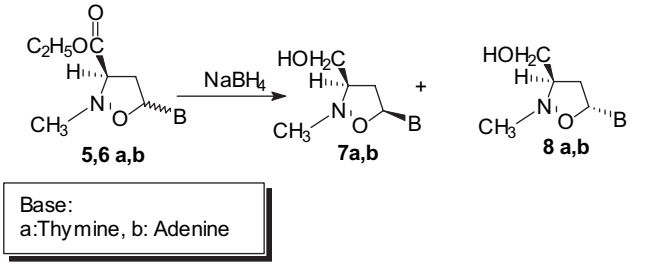
The molecular structure of the reaction products was determined on the basis of analytical and spectroscopic data. The isoxazolidine as an epimeric mixture **3, 4** was then coupled with silylated thymine and silylated adenine in CH_2Cl_2 and then trimethylsilyltriflate was added, the reaction mixture was refluxed for 1 h, then cooled to 0°C, affording nucleosides **5a,b** and **6a,b** as epimeric mixture in a good overall yield (60%) as shown in Scheme 2. The epimeric mixtures of compounds **5a,b** and **6a,b** were then treated with $NaBH_4$ in a 1:5 ratio in (dioxane:water 1:1) as solvents at room temperature affording, in high yields, nucleosides **7a,b** and **8a,b**, respectively, as shown in Scheme 3.



Scheme 1: Reaction of nitrone 2 with vinylacetate.



Scheme 2: Reaction of isoxazolidine 3,4 with nitrogen bases.



Scheme 3: Reduction of nucleoside 5,6 a,b with NaBH4.

Biological Activity

The Spot Test

In the spot test, most compounds can be applied directly on the agar surface. As the test compound diffuses out from the central spot, a range of concentrations is tested simultaneously (Maron and Ames, 1983).

This test was carried out initially to qualitatively determine the dose range of toxicity and/or mutagenicity that may be exhibited by **7, 8a** and **8b**. No inhibition zone was observed in all strains indicating a lack of toxicity in the tested dose range. Tables 1 and 2 summarize the results of this test for **7, 8a** and **8b**; +=20–100; ++=100–200; +++=200–500; ++++=>500; and −=<20. In general, the initial assessment of the genotoxic potential of **7, 8a** and **8b** in the Ames *Salmonella* spot test revealed that in the absence and presence of metabolic activation, **7, 8a** and **8b** induced about (20–100) his+revertants in all strains. However, it should be clarified that a positive mutagenic activity of a chemical compound in the spot test is indicated by clustering of his revertants with or without the presence of an inhibition zone. Such a result could not be observed for the two compounds suggesting that the two compounds are nonmutagenic. Furthermore, the use of S9 mix did not convert the studied compounds into mutagenic ones, although there was an increase in the number of revertants. It is worth mentioning that the presence of 20–100 revertant colonies over the control values in spot test is of no value concerning any decision about the mutagenicity of a chemical compound. The spot test is less sensitive than

Table 1: Reversion of *Salmonella typhimurium* tester strains with **7, 8a** with and without S9 mix.

		Average number of revertant colonies/plate[a]					
		TA97		TA100		TA102	
Compound	Dose (μg/plate)	–S9	+S9	–S9	+S9	–S9	+S9
7, 8a	300	154	148	112	118	275	370
	100	161	193	134	129	395	405
	33.33	170	181	138	176	401	414
	11.11	187	163	126	163	422	399
	3.70	203	196	143	162	425	437
	1.23	210	200	169	188	467	486
	0.41	180	204	174	148	417	385
	0.14	159	195	137	168	377	395
DMSO	100 μL	165	159	125	140	380	415
SA	1.5	–	–	1400	14800	–	–
NPD	20	980	870	–	–	–	–
MMC	0.5	–	–	–	–	2300	2250

DMSO, dimethyl sulfoxide; SA, sodium azide; NPD, 4-nitro-*o*-phenylenediamine; MMC, mitomycin C.
[a]Each number represents a mean value from at least two separate experiments.

Table 2: Reversion of *Salmonella typhimurium* tester strains with **8b** with and without S9 mix.

		Average number of revertant colonies/plate[a]					
		TA97		TA100		TA102	
Compound	Dose (μg/plate)	–S9	+S9	–S9	+S9	–S9	+S9
8b	300	130	155	138	198	310	398
	100	140	175	162	150	423	376
	33.33	185	215	200	156	390	400
	11.11	205	232	152	172	415	396
	3.70	168	210	195	174	461	434
	1.23	194	207	139	145	370	390
	0.41	183	188	178	168	444	478
	0.14	241	195	180	167	412	429
DMSO	100 μL	165	159	125	140	380	415
SA	1.5	–	–	1400	14800	–	–
NPD	20	980	870	–	–	–	–
MMC	0.5	–	–	–	–	2300	2250

DMSO, dimethyl sulfoxide; SA, sodium azide; NPD, 4-nitro-*o*-phenylenediamine; MMC, mitomycin C.
[a]Each number represents a mean value from at least two separate experiments.

the standard plate incorporation assay as only relatively few bacteria on the plate are exposed to the chemical at any particular dose level. Therefore, the ability of **7, 8a** and **8b** to induce revertants in the four strains was further analyzed using the standard plate incorporation and the preincubation assays.

The Standard Plate Incorporation Assay and the Preincubation Assay

The results of these mutagenicity assays in the presence and absence of S9 mixture are shown in Tables 1 and 2. More than twofold increase in the number of revertants over the number of spontaneous revertants is considered as a positive result (Maron and Ames, 1983; Ono et al., 1994).

Both derivatives showed no mutagenic activity in the three *Salmonella* strains without metabolic activation. Bacterial background lawn examination revealed no toxicity of **7, 8a** and **8b** in the tested dose range. No dose–response increase could be detected. The response was negative and the compounds were retested using the preincubation procedure with S9 mix.

The use of the S9 mix did not convert the tested compounds into mutagens, although the used induction procedure by phenobarbital and β-naphthoflavone produced induction similar to Aroclor-1254, which is potent against polycyclic hydrocarbons and aromatic amines (Matsushima et al., 1976). Because the tested compounds induced a number of revertants that did not reach double the number of spontaneous revertants using solvent control, they were considered to be nonmutagenic in the three tested *Salmonella* strains and so they can be classified as causing neither base-pair substitutions

in TA100 nor frameshift mutations in TA97. The negative results detected with strain TA102 indicate that the compounds are not oxidative mutagens.

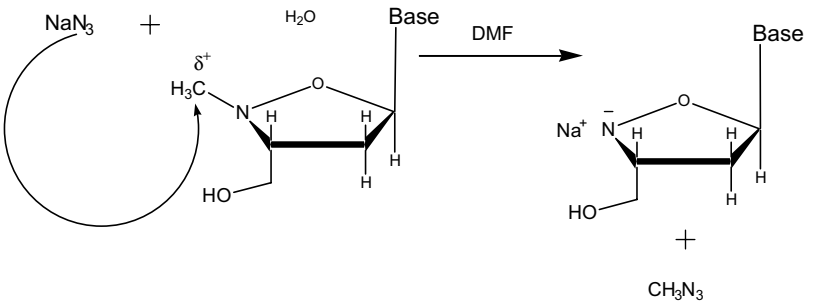
At the highest used concentration, namely 300 μg/plate **7, 8a** and **8b** did not inhibit the growth of bacterial cells. This means that they are not toxic at such concentration. In general, the effects of **7, 8a** and **8b** on the standard mutagens, namely NPD, MMC, and hydrogen peroxide, were similar. Both compounds showed no mutagenic repression activity against hydrogen peroxide and mitomycin C using TA102 or against NPD using TA97. This implies that both compounds could not act as desmutagens against hydrogen peroxide, mitomycin C, and NPD. However, the tested compounds showed a detectable and notable antimutagenic activity against sodium azide using strain TA100. This last strain is known as a base-pair substitution detector strain. The percent mutagenic repression at 300 μg/plate, which is the highest concentration used for both **7, 8a** and **8b** compounds, was 82.70% and 79.10%, respectively (Table 3). Such results may indicate that neither **7, 8a** nor **8b** were able to act as (1) inhibitors of metabolic activation of H_2O_2, MMC, or NPD *in vitro*; (2) inactivators of the above mutagens themselves, or (3) interfering agents with the DNA repair systems.

Concerning SA, although other mechanisms of action of the investigated compounds as antimutagenic substances cannot be totally excluded, but taking into consideration that the preincubation method was followed, we suggest a mechanism by which 7, 8a and 8b may act as desmutagens against such a mutagenic compound (Scheme 4). As can be seen in this scheme, the N-methyl nucleoside

Table 3: Antimutagenic activity of **7, 8a** and **8b** in *Salmonella typhimurium* tester strain TA100.

Compound	Dose (μg/plate of nucleoside analogue)	Average number of revertant colonies/plate[a]	Percent mutagenic repression (%)
DMSO	100 (μL)	166	–
7, 8a	300	374	82.70
	100	395	80.90
	33.33	630	61.46
	11.11	757	50.91
	3.7	803	47.10
	1.23	982	32.22
	300[b]	154	–
8b	300	418	79.10
	100	450	76.41
	33.33	571	66.36
	11.11	900	39.00
	3.7	1014	29.57
	1.23	1246	10.30
	300[b]	177	–
SA	5	1370	–

DMSO, dimethyl sulfoxide; SA, sodium azide.
[a]Mean values from at least three plates/dose and two separate experiments.
[b]Chemical control (Nucleoside analogue without the authentic mutagen).



Scheme 4: Suggested mechanism for the antimutagenic activity of compounds 7ab and 8b against SA.

7a,b and 8b possess in their structure an electrophilic center represented by the N-methyl group that is considered a valid candidate to react with sodium azide in DMF through a nucleophilic substitution type SN2. This may shed some light that may help in the explanation of the antimutagenic activity detected in the current investigation. Surveying the literature, one can find a wide range of nucleoside derivatives that possess antimutagenic properties. Just to present some examples, purine ribosides such as adenosine decreased the synergistic mutagenic activity of puromycin aminonucleoside and UV (Sideropoulos, 1979). 5-Fluorouracil and 5-fluorodeoxyuridine were also found to inhibit considerably the UV induction of the SOS gene (Ohta et al., 1986). Antimutagenic effects of 2′-deoxyuridines were discovered: in V79 cells, 5-(2-bromovinyl)-2′-deoxyuridine, 5-ethyl-2′-deoxyuridine, 2′-deoxyuridine, and 2′-deoxythymidine prevented the mutagenicity induced by 5-(2-chloroethyl)-2′-deoxyuridine; in these cells, 5-ethyl-2′-deoxyuridine also inhibited the mutagenicity induced by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG). In primary rat hepatocytes, 5-iodo-2′-deoxyuridine and 5-ethyl-2′-deoxyuridine inhibited the induction of unscheduled DNA synthesis induced by MNNG or UV light (Marquardt et al., 1988). Furthermore, it was found that frameshift mutagenesis induced by 9-aminoacridine was significantly decreased in both Salmonella typhimurium and Escherichia coli when high concentrations of cAMP were added to the defined medium. Other adenosine 5′-phosphates such as adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), and adenosine 5′-monophosphate (AMP) added to the liquid medium during 9-aminoacridine treatment also substantially decreased the reversion rate to prototrophy in the aforementioned strains, as did adenosine itself (Kopsidas and MacPhee, 1996). Pseudouridine was shown to have an antimutagenic property. It showed an antimutagenic activity against MNNG in a dose-dependent manner. Also, it was found to inhibit bacterial mutagenicity of another methylating agent, N-methyl-N-nitrosourea. Furthermore, among analogues of pseudouridine, spongouridine was antimutagenic against MNNG (Yoshikawa et al., 2002). As the synthesized compounds in the current investigation were found to be nonmutagenic and with antimutagenic properties, further studies of their biological activities including anti-HIV and anticarcinogenicity are highly recommended.

CONCLUSION

Novel nucleoside derivatives were successfully synthesized as biologically active compounds, for example as anti-HIV agents. The compounds were found to be nonmutagenic using the Ames test. However, testing them for antimutagenic properties revealed that they are active against the base-pair substitution mutagen sodium azide. On the other hand, they did not show any indication as antimutagenic agents against hydrogen peroxide and mitomycin C (oxidative mutagens) or against nitrophenylenediamine (both frameshift and base-pair substitution mutagens).

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