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2012 Ara Yardım

Tarananlar: {'journal', 'of', 'biochemical', 'and', 'molecular', 'toxicology'}@Anahtar;
{'2012'}@Yayın Yılı, Sayfa: 1

No	Dergi Adı	Dergi Kısa Adı	ISSN	Dergi Grubu	Yayın Yılı	İndeks Türü
1.	JOURNAL OF BIOCHEMICAL AND MOLECULAR TOXICOLOGY	J BIOCHEM MOL TOXIC	1095-6670	C	2012	Fen (SCI)

Antigenotoxic Properties of Two Newly Synthesized β -Aminoketones Against *N*-Methyl-*N'*-Nitro-*N*-Nitrosoguanidine and 9-Aminoacridine-Induced Mutagenesis

S. Arda Ozturkcan,¹ Kadir Turhan,¹ Zuhale Turgut,¹ Mehmet Karadayi,² and Medine Gulluce²

¹Department of Chemistry, Yildiz Technical University, Istanbul, Turkey

²Department of Biology, Atatürk University, Erzurum, Turkey; E-mail: mkaradayi@atauni.edu.tr

Received 1 February 2012; revised 21 March 2012; accepted 14 April 2012

ABSTRACT: The aim of this study was to determine the antigenotoxic potential of two newly synthesized β -aminoketones against *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and 9-aminoacridine (9-AA)-induced mutagenesis. The mutant bacterial tester strains were MNNG-sensitive *Escherichia coli* WP2uvrA and 9-AA-sensitive *Salmonella typhimurium* TA1537. Both test compounds showed significant antimutagenic activity at various tested concentrations. The inhibition rates ranged from 29.5% (compound 1: 2 mM/plate) to 47.5% (compound 2: 1.5 mM/plate) for MNNG and from 25.0% (compound 2: 1 mM/plate) to 52.1% (compound 2: 2.5 mM/plate) for 9-AA genotoxicity. Moreover, the mutagenicity of the test compounds was investigated by using the same strains. Neither test compound has mutagenic properties on the bacterial strains at the tested concentrations. Thus, the findings of the present study give valuable information about chemical prevention from MNNG and 9-AA genotoxicity by using synthetic β -aminoketones. © 2012 Wiley Periodicals, Inc. *J Biochem Mol Toxicol* 26:258–263, 2012; View this article online at wileyonlinelibrary.com. DOI 10.1002/jbt.21414

KEYWORDS: β -Aminoketones; Mutagenicity; Antimutagenicity; *E. coli* WP2 Test; Ames/*Salmonella* Test

INTRODUCTION

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and 9-aminoacridine (9-AA) are two well-known genotoxic agents and have wide effects on many living organisms. MNNG is a well-known carcinogen and has been used for ages as a chemical methylating agent that causes DNA damages. Furthermore, it has been recommended as one of the best model chemicals for scientific studies, focusing on exploring pathways of DNA repair, DNA damage response, and mutagenesis [1]. On the other hand, 9-AA is a member of acridine family, known as model frameshift mutagen and frequently used in bacterial assay systems [2]. In the mutagenesis mechanism, 9-AA binds to DNA noncovalently by intercalation that frequently results in frameshift mutagenesis [3]. Thus, 9-AA has been utilized as a model chemical to investigate potential hazards of various intercalating agents and formation of related disorders in living organisms.

To date, many studies have focused on MNNG and 9-AA genotoxicity to determine their effective mechanisms and prevention perspectives because of their wide-range of hazardous potential [1–9]. In this manner, a combination of natural or synthetic heterocyclic compounds and effective genotoxicity test systems has a great importance and potential to develop preservative agents against mutagens and their negative effects [10,11].

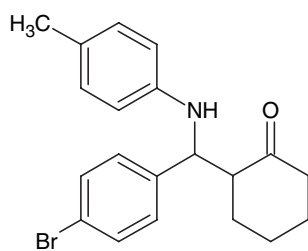
β -Aminoketones, also known as Mannich base derivatives, are important heterocyclic compounds. Owing to various effects on living organisms, there has been a growing interest in the synthesis of novel β -aminoketones and determination of their biological activities [12–18].

Correspondence to: Mehmet Karadayi.

Contract Grant Sponsor: Yildiz Technical University.

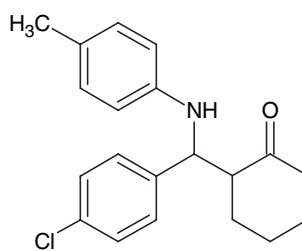
Contract Grant Number: BAPK 2010-01-02-DOP01.

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1

2-((4-Bromophenyl)((4-methylphenyl)amino)methyl)cyclohexanone



2

2-((4-Chlorophenyl)((4-methylphenyl)amino)methyl)cyclohexanone

FIGURE 1. Two newly synthesized β -aminoketones: 2-((4-bromophenyl)((4-methylphenyl)amino)methyl)cyclohexanone and 2-((4-chlorophenyl)((4-methylphenyl)amino)methyl)cyclohexanone.

Thus, the present study was designed to evaluate the mutagenic and antimutagenic potentials of two newly synthesized β -aminoketones (Figure 1) by using Ames/*Salmonella* and *Escherichia coli* WP2 bacterial reverse mutation assay systems.

MATERIALS AND METHODS

Chemicals

Direct acting mutagens 9-AA and MNNG were obtained from Sigma-Aldrich (St. Louis, MO) and ABCR GmbH & Co. KG (Karlsruhe, Germany), respectively. Other solvents and pure chemicals including magnesium sulfate (MgSO_4), sodium ammonium phosphate ($\text{Na}_2\text{NH}_2\text{PO}_4$), D-glucose, D-biotin, sodium chloride (NaCl), L-histidine HCl, L-tryptophane, sodium phosphate-dibasic (Na_2HPO_4), crystal violet, citric acid monohydrate, potassium phosphate-dibasic (K_2HPO_4), and sodium phosphate-monobasic (NaH_2PO_4) were also obtained from Difco (Franklin Lakes, NJ), Fluka (Steinheim, Germany), Merck (Darmstadt, Germany), and Sigma (St. Louis, MO).

Synthesis Method for β -Aminoketones

To a solution of $\text{Bi}(\text{OTf})_3 \cdot 4\text{H}_2\text{O}$ (0.05 mmol) in 1 mL of water, aldehyde (1 mmol) (thiophene-2-carbaldehyde and 4-nitrobenzaldehyde), aniline (1 mmol), and cyclohexanone (2 mmol) were added. The reaction mixture was stirred vigorously with a magnetic stirrer during the mentioned time at room temperature and then stopped by the addition of ethyl acetate (EtOAc). The aqueous phase was extracted with EtOAc (3×10 mL). The organic phases were combined and washed with water (2 mL) and brine (2 mL) consecutively, dried over MgSO_4 , and filtered. The solvents were removed under reduced pressure. The crude mixture was purified by column chromatography (hexane/EtOAc 3:1) [19].

Bacterial Tester Strains

E. coli WP2 *uvrA* (ATCC[®] number: 49979) strain was provided by LGC standards (Middlesex, UK), and *Salmonella typhimurium* TA1537 (ATCC[®] Number: 29630) strain was provided by The American Type Culture Collection – Bacteria Department of Georgetown University, Washington, DC. All strains were stored at 80°C. Working cultures were prepared by inoculating nutrient broth with the frozen cultures, followed by an overnight incubation at 37°C with gentle agitation [20].

Viability Assay and Determination of Test Concentrations

The toxicity of chemicals toward *E. coli* WP2 *uvrA* and *S. typhimurium* TA1537 strains was determined as described in detail elsewhere [21,22]. These tests confirmed that there was normal growth of the background lawn, spontaneous colony numbers within the regular range, and no significant reduction in cell survival. Thus, for the concentrations and conditions reported here, no toxicity or other adverse effects was observed.

Bacterial Reverse Mutation Assay

The bacterial mutagenicity and antimutagenicity assays were performed as described in detail elsewhere [23,24]. The known mutagens MNNG [in 10% dimethyl sulfoxide (DMSO): 1 $\mu\text{g}/\text{plate}$] for *E. coli* WP2 *uvrA* and 9-AA (in methanol: 40 $\mu\text{g}/\text{plate}$) for *S. typhimurium* TA1537 were used as positive controls. 10% DMSO was also used as negative control in these studies.

In the mutagenicity test performed with TA1537 strain of *S. typhimurium*, 100 μL of the overnight bacterial culture, 50 μL of the test compounds at different concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mM/plate in 10% DMSO), and 500 μL of buffer solution were added to 2 mL of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose agar plates. Histidine-independent revertant

colonies and viable cells were scored on plates after incubation at 37°C for 48 h.

In the antimutagenicity test performed with the same strain, 100 µL of the overnight bacterial culture, 50 µL of mutagen solution, and 50 µL of the test compounds at different concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mM/plate in 10% DMSO), and 500 µL of buffer were added to 2 mL of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose agar plates. Histidine-independent revertant colonies and viable cells were scored on plates after incubation at 37°C for 48 h.

The procedures of mutagenicity and antimutagenicity assays described for the Ames/*Salmonella* test are all applicable to the *E. coli* WP2 reverse mutation assay. The only procedural difference is the addition of limited tryptophan (0.01 mM) instead of histidine to the top agar [23].

The plate incorporation method was used to assess the results of mutagenicity and antimutagenicity assays [25].

In mutagenicity assays, the mutagenic index was calculated for each concentration, which is the average number of revertants per plate divided by the average number of revertants per plate with the negative (solvent) control. A sample is considered mutagenic when a dose-response relationship was observed, and a twofold increase in the number of mutants with at least one concentration was observed [10,26].

In antimutagenicity assays, the inhibition of mutagenicity was calculated by using the following equation (where M : the number of revertants/plate induced by

mutagen alone, S_0 : the number of spontaneous revertants, S_1 : the number of revertants/plate induced by the test compound plus the mutagen):

$$\% \text{Inhibition} = 1 - [(M - S_1)/(M - S_0)] \times 100.$$

25%–40% inhibition was defined as moderate antimutagenicity, 40% or more inhibition as strong antimutagenicity, and less than 25% inhibition as no antimutagenicity [27].

Statistical Analysis

The results are presented as the average and standard error of three experiments with triplicate plates/dose experiment. The data were further analyzed for statistical significance using analysis of variance, and the difference among means was compared by a high-range statistical domain using Tukey's test. A level of probability was taken as $P < 0.05$ indicating statistical significance [10].

RESULTS

According to the mutagenicity assay results, neither test compound has mutagenic activity on the mutant bacterial tester strains *E. coli* WP2*uvrA* and *S. typhimurium* TA1537 at the tested concentrations (Table 1).

The possible antimutagenic potentials of the test compounds were also examined against MNNG and 9-AA mutagenicity on *E. coli* WP2 *uvrA* and *S. typhimurium* TA1537 strains, respectively. The results of

TABLE 1. Mutagenicity Results of Two Newly Synthesized β -Aminoketones

Test Items	Concentration (mM/plate)	Number of Revertants			
		<i>E. coli</i> WP2 <i>uvrA</i>		<i>S. typhimurium</i> TA1537	
		Mean \pm Standard Error	Mutagenicity (%)	Mean \pm Standard Error	Mutagenicity (%)
Compound 1 (C ₂₀ H ₂₂ BrNO)	0.5	33.33 \pm 1.33	–	25.66 \pm 0.95	–
	1.0	34.66 \pm 1.11	–	24.50 \pm 1.02	–
	1.5	33.83 \pm 1.55	–	25.00 \pm 1.34	–
	2.0	32.66 \pm 0.66	–	23.66 \pm 0.76	–
	2.5	33.50 \pm 1.33	–	28.00 \pm 0.51	–
Compound 2 (C ₂₀ H ₂₂ ClNO)	0.5	33.00 \pm 1.36	–	26.50 \pm 1.28	–
	1.0	35.83 \pm 0.54	–	25.83 \pm 1.07	–
	1.5	33.33 \pm 0.91	–	24.50 \pm 1.36	–
	2.0	34.66 \pm 1.35	–	25.00 \pm 0.57	–
	2.5	34.33 \pm 1.38	–	25.50 \pm 1.60	–
MNNG		363.16 \pm 8.95			
9-AA				645.33 \pm 11.49	
DMSO (µL/plate)		34.66 \pm 1.35		26.16 \pm 1.13	

MNNG (1 µg/plate) and 9-AA (40 µg/plate) were used as positive controls for *E. coli* WP2 *uvrA* and *S. typhimurium* TA1537 strains, respectively. DMSO (dimethylsulfoxide; 100 µL/plate) was used as negative control.

TABLE 2. Antimutagenicity Results of Two Newly Synthesized β -Aminoketones

Test Items	Concentration (mM/plate)	Number of revertants			
		<i>E. coli</i> WP2 <u>uvrA</u>		<i>S. typhimurium</i> TA1537	
		Mean \pm Standard Error	Inhibition (%)	Mean \pm Standard Error	Inhibition (%)
Compound 1 (C ₂₀ H ₂₂ BrNO)	0.5	240.00 \pm 08.73	30.6*	381.00 \pm 10.65	37.9*
	1.0	309.00 \pm 09.03	10.7	321.50 \pm 07.60	47.6*
	1.5	281.50 \pm 07.29	18.6	312.50 \pm 07.33	49.1*
	2.0	244.00 \pm 08.18	29.5*	394.00 \pm 08.88	35.8*
	2.5	295.50 \pm 08.84	14.6	352.00 \pm 09.11	42.7*
Compound 2 (C ₂₀ H ₂₂ ClNO)	0.5	229.50 \pm 06.55	33.7*	614.16 \pm 10.49	-
	1.0	235.50 \pm 09.26	31.9*	460.50 \pm 11.66	25.0*
	1.5	181.50 \pm 09.24	47.5*	327.50 \pm 08.26	46.7*
	2.0	229.00 \pm 09.79	33.8*	317.50 \pm 10.95	48.3*
	2.5	237.00 \pm 07.14	31.5*	294.00 \pm 08.91	52.1*
MNNG		346.00 \pm 8.53			
9-AA				614.00 \pm 8.48	
DMSO (μ L/plate)		34.50 \pm 1.28		25.83 \pm 1.60	

MNNG (1 μ g/plate) and 9-AA (40 μ g/plate) were used as positive controls for *E. coli* WP2uvrA and *S. typhimurium* TA1537 strains, respectively.

DMSO (dimethylsulfoxide; 100 μ L/plate) was used as negative control.

*Statistically significant difference from control ($P < 0.05$).

the antimutagenicity assay were evaluated by using the standard plate incorporation method and are summarized in Table 2, showing the antimutagenic activities of the test compounds tested at five different concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mM/plate).

In the antimutagenicity assay performed with *E. coli* WP2 *uvrA* strain, both test compounds showed antimutagenic activity against MNNG mutagenicity at different test concentrations (Table 2). The significant inhibition rates of MNNG mutagenicity ranged from 29.5% (compound 1: 2 mM/plate) to 47.5% (compound 2: 1.5 mM/plate).

Furthermore, both test compounds also exhibited significant antimutagenic activities on 9-AA-induced mutagenesis in the *S. typhimurium* TA1537 strain at all tested concentrations, except 0.5 μ M/plate concentration of compound 2 (Table 2). The inhibition rates were between 25.0% (compound 2: 1 mM/plate) and 52.1% (compound 2: 2.5 mM/plate).

DISCUSSION

The synthesis and determination of biological activities of various compounds are very important strategies owing to their large-scale usefulness in industrial and medicinal areas [10,28,29]. In this regard, synthetic β -aminoketones, which is a Mannich base derivative formed in the reaction of an amine, formaldehyde (or an aldehyde), and a carbon acid, have a great potential to be candidates for industrial

and medicinal applications. The Mannich reaction has been one of the classical methods for the construction of nitrogenous compounds especially β -aminocarbonyl compounds that are versatile intermediates for the synthesis of β -amino alcohols and acids, which have a great deal of biological significance [12]. β -Aminocarbonyl derivatives are an important class of aromatic compounds that occur in various pharmaceuticals, natural products, and versatile synthetic intermediates [13,14], and recent studies showed important biological properties of aminoketone derivatives including antitubercular, antifungal, antimalarial, antidiabetic, anti-inflammatory, and antitumor activities [15–18]. Thus, the mutagenic and antimutagenic properties of two newly synthesized Mannich bases have been investigated in the current study by using *E. coli* WP2 and Ames/*Salmonella* test systems.

The results obtained from the mutagenicity assays showed that the test materials (compounds 1 and 2) have no mutagenic activity on *E. coli* WP2uvrA and *S. typhimurium* TA1537 strains at all tested concentrations.

In the antimutagenicity assays, it was shown that compounds 1 and 2 have antimutagenic activity on chemical-induced mutagenesis in *E. coli* WP2uvrA and *S. typhimurium* TA1537 strains at various tested concentrations including 0.5, 1, 1.5, 2, and 2.5 mM/plate.

The mutagens used to determine the antimutagenic activity of the test substances in this study were MNNG for WP2uvrA and 9-AA for TA1537.

MNNG is a well-known carcinogen, and it is known to exert its mutagenic and lethal effects by

methylation of DNA [10]. Previous studies showed that the formation of O⁶-methylguanine, which is one of its important products, appears to be responsible for its mutagenic action [4,5]. The results of this study showed that both test compounds have antimutagenic activity against MNNG at tested concentrations. The antimutagenicity of these substances may be explained on the basis of their inhibitor activity on the production of O⁶-methylguanine.

9-AA was used in this study as a simple intercalator mutagen. Through intercalation, 9-AA induces frameshift mutations at hotspots in which a single base, especially guanine, is repeated [3,7]. The antimutagenicity assay performed with *S. typhimurium* TA1537 and 9-AA depends on the inhibition of this mechanism by test substances, which were thought as antimutagenic. In this study, the results suggested that compounds 1 and 2 have antimutagenic activity in TA1537 strain at different concentrations (Table 2). This antimutagenic effect may be due to their inhibition capabilities by blocking 9-AA binding to DNA.

CONCLUSIONS

In conclusion, both test compounds investigated in the present study can be considered genotoxically safe at the tested concentrations and they also provided important antimutagenic properties. These activities are valuable for further investigations on protective strategy development against MNNG- and 9-AA-induced genotoxicity. The data obtained from the present study can also be supported by performing complicate test systems, resulting in more reliable results that will be applicable to human health.

REFERENCES

- Wyatt MD, Pittman DL. Methylating agents and DNA repair responses: methylated bases and sources of strand breaks. *Chem Res Toxicol* 2006;19:1580–1594.
- Ferguson LR, Denny WA. The genetic toxicology of acridines. *Mutat Res* 1991;258:123–160.
- Hoffmann GR, Calciano MA, Lawless BM, Mahoney KM. Frameshift mutations induced by three classes of acridines *lacZ* reversion assay in *Escherichia coli*: potency of responses and relationship to slipped mispairing models. *Environ Mol Mutagen* 2003;42:111–121.
- Loveless A. Possible relevance of O-6 alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides. *Nature* 1969;223:206–208.
- Eadie JS, Conrad M, Toorchen D, Topal MD. Mechanism of mutagenesis by O⁶-methylguanine. *Nature* 1984;308:201–203.
- Neidle S, Abraham Z. Structural and sequence-dependent aspects of drug intercalation into nucleic acids. *CRC Crit Rev Biochem* 1984;17:73–121.
- Ferguson LR, Denny WA. Frameshift mutagenesis by acridines and other reversibly-binding DNA ligands. *Mutagenesis* 1990;5:529–540.
- Bailey C, Denny WA, Mellor LE, Wakelin LPG, Waring MJ. Sequence specificity of the binding of 9-aminoacridine- and amsaacridine-4-carboxamides to DNA studied by DNase I footprinting. *Biochemistry* 1992;31:3514–3524.
- Kumaresan KR, Springhorn SS, Lacks SA. Lethal and mutagenic actions of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine potentiated by oxidized glutathione, a seemingly harmless substance in the cellular environment. *J Bacteriol* 1995;177:3641–3646.
- Gulluce M, Agar G, Baris O, Karadayi M, Orhan F, Sahin F. Mutagenic and antimutagenic effects of some *Astragalus* species grown in the eastern Anatolia region of Turkey. *Phytother Res* 2010;24:1014–1018.
- Guvenalp Z, Gulluce M, Karadayi M, Ozbek H, Arasoglu T, Demirezer LO. Determination of mutagenic and antimutagenic properties of flavonoid compounds isolated from *Mentha longifolia* ssp. *longifolia* and *Origanum vulgare* ssp. *vulgare* by using AMES test system. *Planta Med* 2010;76:1286–1287.
- Shailaja M, Manjula A, Vittal Lao B. Three component one pot synthesis of β -amino ketones mediated by (bromodimethylsulfonium) bromide. *Indian J Chem B* 2010;49:482–486.
- Tramontini M, Angiolini L. Further advances in the chemistry of mannich bases. *Tetrahedron* 1990;46:1791–1837.
- Yi L, Zou JH, Lei HS, Lin XM, Zhang MX. The Mannich reaction of cyclic-ketones, aromatic-aldehydes and aromatic-amines. *Org Prep Proc Int* 1991;23:673–675.
- Witt A, Bergman J. Recent developments in the field of quinazoline chemistry. *Curr Org Chem* 2003;7:659–677.
- Ma ZZ, Hano YS, Nomura T, Chen YJ. Novel quinazoline-quinoline alkaloids with cytotoxic and DNA topoisomerase II inhibitory activities. *Bioorg Med Chem Lett* 2004;14:1193–1196.
- Connolly DJ, Cusack D, O'Sullivan TP, Guiry PJ. Synthesis of quinazolinones and quinazolines. *Tetrahedron* 2005;61:10153–10202.
- Mhaske SB, Argade NP. The chemistry of recently isolated naturally occurring quinazolinone alkaloids. *Tetrahedron* 2006;62:9787–9826.
- Ollevier T, Nadeau E, Guay-Bégin AA. Direct-type catalytic three-component Mannich reaction in aqueous media. *Tetrahedron Lett* 2006;47:8351–8354.
- Oh HT, Kim SH, Choi HJ, Chung MJ, Ham SS. Antioxidative and antimutagenic activities of 70% ethanol extract from masou salmon (*Oncorhynchus masou*). *Toxicol In Vitro* 2008;22:1484–1488.
- Santana-Rios G, Orner GA, Amantana A, Prowost C, Wu SY, Dashwood RH. Potent antimutagenic activity of white tea in the *Salmonella* assay. *Mutat Res* 2001;495:61–74.
- Yu Z, Xu M, Santana-Rios G, Shen R, Izquierdo-Pulido M, Williams DE, Dashwood RH. A comparison of whole wheat, refined wheat and wheat bran as inhibitors of heterocyclic amines in the *Salmonella* mutagenicity assay and in the rat colonic aberrant crypt focus assay. *Food Chem Toxicol* 2001;39:655–665.

23. Mortelmans K, Riccio ES. The bacterial tryptophan reverse mutation assay with *Escherichia coli* WP2. *Mutat Res* 2000;455:61–69.
24. Mortelmans K, Zeiger E. The Ames *Salmonella*/microsome mutagenicity assay. *Mutat Res* 2000;455:29–60.
25. Maron DM, Ames BN. Revised methods for the *Salmonella* mutagenicity test. *Mutat Res* 1983;113:173–215.
26. Santos FV, Tubaldini FR, Cólus IMS, Andréo MA, Bauab TM, Leite CQF, Vilegas W, Varanda EA. Mutagenicity of *Mouriri pusa* Gardner and *Mouriri elliptica* Martius. *Food Chem Toxicol* 2008;46:2721–2727.
27. Evandri MG, Battinelli L, Daniele C, Mastrangelo S, Bolle P, Mazzanti G. The antimutagenic activity of *Lavandula angustifolia* (lavender) essential oil in the bacterial reverse mutation assay. *Food Chem Toxicol* 2005;43:1381–1387.
28. Avunduk S, Mitaine-Offer AC, Alankus-Caliskan O, Miyamoto T, Senol SG, Lacaille-Dubois MA. Triterpene glycosides from the roots of *Astragalus flavescens*. *J Nat Prod* 2008;71:141–145.
29. Agar G, Gulluce M, Aslan A, Bozari S, Karadayi M, Orhan F. Mutation preventive and antigenotoxic potential of methanol extracts of two natural lichen. *J Med Plants Res* 2010;4(20):2132–2137.