Gazi University Journal of Science
GU J Sci
25(1):1-8 (2012)

ORIGINAL ARTICLE

Mutagenicity of 1-Ethyl-2,4,5-triphenyl-1H-imidazole and Six Derivatives in *Salmonella typhimurium*

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Received: 03.12.2010 Revised: 27.02.2011 Accepted: 27.09.2011

ABSTRACT

Newly synthesized 1-Ethyl-2,4,5-triphenyl-1H-imidazole and its six derivatives were tested by Ames assay. In order to reveal the mutagenic activities of the compounds, two different mutant strains of *Salmonella typhimurium* (TA98 and TA100) were used in an Ames assay with/without S9 microsomal fraction from rat liver. It was found that the compounds have no mutagenic activities.

Key Words: *1-Ethyl-2,4,5-triphenyl-1H-imidazole, Ames/Salmonella/Microsome assay, mutagenicity, predrug.*

1. INTRODUCTION

Over the past century a lot of new chemicals have been synthesized for different purposes. Humans and all kinds of living organisms may have been exposed to these compounds at any time [1]. Some of the compounds have been reported as mutagens which can cause mutations and fertility problems [2]. Although all of the new compounds have not been investigated for their hazardous potentials, as a way of safeguarding people some research centres have routinely tested many of them for their toxic and mutagenic activities [3]. One of these centres is the International Agency for Research on Cancer (IARC) which classifies chemical compounds according to their *in vitro* or *in vivo* mutagenic activities [4].

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It is vital to test the mutagenic activities of new compounds because they may cause genetic changes like single base mutations (substitution, insertion or deletion), chromosome breaks or losses which could lead to genetic diseases and cancer in living organisms. The Ames/Salmonella/Microsome test system is the most widely accepted short term mutagenicity test. This test system has been used especially for the determination of a chemical compound's particular mutagenic effects [2].

In the literature there are thousands of reports which mainly use the Ames assay to determine the mutagenicity of number of different kinds of chemicals and materials such as heterocyclic aromatic amines [5, 6], hexamethylphosphoramide [7], some ionic liquids like imidazolium, pyridinium, ammonium [8], phenyltetrahydropyridinyl butylazole [9], settled house dust [10], drinking water [11], surface water [12], textile dyes [13], organic aerosol pollutants [14], and plant extracts [15]. Furthermore, the Ames assay has been used for anti-mutagenicity as well as mutagenicity [16].

Imidazole is an organic compound and one of the important groups of heterocyclic aromatic amines [17]. It has been reported that imidazole derivatives have a broad range of biological acitvities and they have very important place in medicinal chemistry [18].

Imidazole and its derivatives are a significant group of heterocyclic aromatic amines [17]. Imidazole derivatives have an important role in medical chemistry and have a wide biological activity range [18]. Imidazole derivatives are known to have various pharmacological effects as analgesics, antiprotozoals, nematosids, tumor inhibitors, sedatives, antiallergics, antiinflammatories, antifungals, and antibacterials [17, 18, 19, 20]. Apart from their different biological activities, some imidazole derivatives have been investigated and their mutagenic potential was indicated by Ames assay [21, 22, 23].

In the present study, the researchers attempted to reveal the mutagenic potential of 1-Ethyl-2,4,5-triphenyl-1H-imidazole and its 6 derivatives through Ames assay.

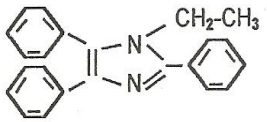
2. MATERIALS AND METHODS

2.1. Chemicals

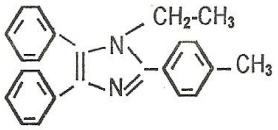
2-aminofluoren, sodium azide (NaN_3), 3-methyl colantren, and sodium phenobarbital were purchased from Fluka, Germany. Commercial D-biotin, ampicillin trihydrate, D-glucose-6-phosphate, and dimethyl sulfoxide were obtained from Merck, Germany. Nutrient broth and agar were sourced from Oxoid, UK.

2.2. Preparation of Test Materials

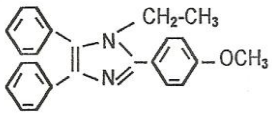
Tested 1-Ethyl-2,4,5-triphenyl-1H-imidazole derivatives were synthesized by Prof. Dr. İlhan IŞIKDAĞ and Assoc. Prof. Dr. Asiye MERİÇ (Anadolu University, Faculty of Pharmacy, Eskişehir, Turkey). The structural forms of the test compounds are given in Figure 1. All test compounds were dissolved in dimethylsulfoxide (DMSO). Cytotoxic doses of the compounds were determined according to Dean et al. (1985) [24].



1-Ethyl-2,4,5-triphenyl-1H-imidazole



1-Ethyl-2-(p-methylphenyl)-4,5-diphenyl-1-H-imidazole



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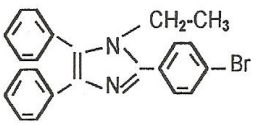

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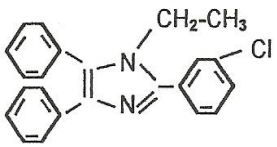

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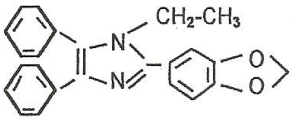

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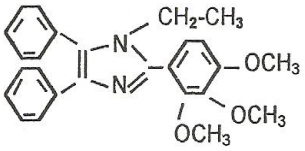


1-Ethyl-2-(o,m,p-trimethoxyphenyl)-4,5-diphenyl-1H-imidazole

Figure 1. 1-Ethyl-2,4,5-triphenyl-1H-imidazole and six derivatives.

2.3. Test Organisms

S. typhimurium TA98 and TA100 mutant strains were obtained from Dr. Bruce Ames (Berkeley, CA, USA). *S. typhimurium* TA98 and TA100 strains are histidine auxotrophs and they could be converted to histidine prototrophs in the presence of certain mutagens.

2.4. Preparation of S9 Fraction

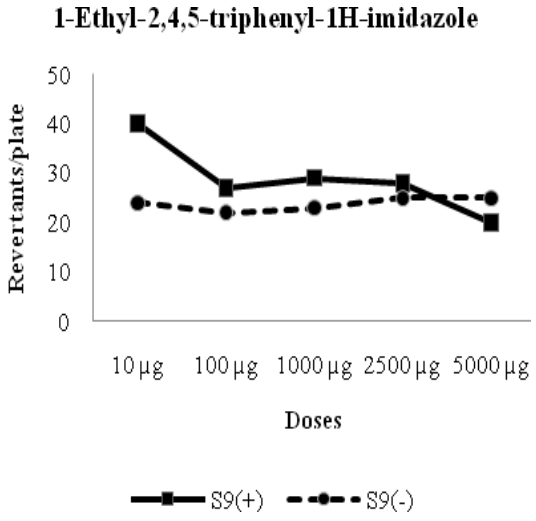
Liver homogenate S9 was used for metabolic activation in the second stage of the Ames assay. The S9 fraction was prepared according to the method described by Garner et al. (1972). For this purpose, Sprague-Dawley male rats (180-200g) were given 3-methyl colantren intraperitoneally (80 mg/kg) during the five days prior to the test. Fenobarbital (0.1 g/l) was given for five days by adding it to drinking water [25].

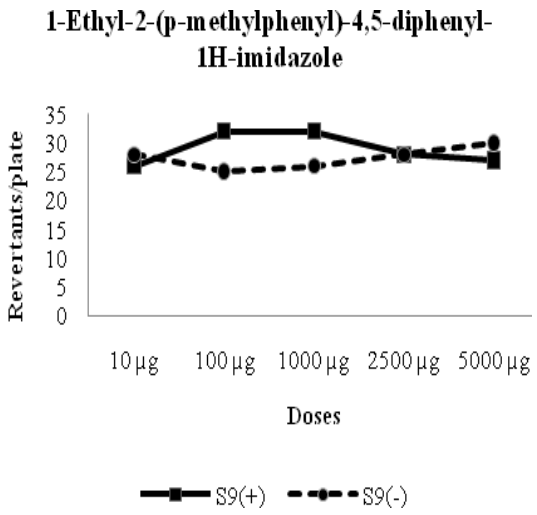
2.5. Mutagenicity Test

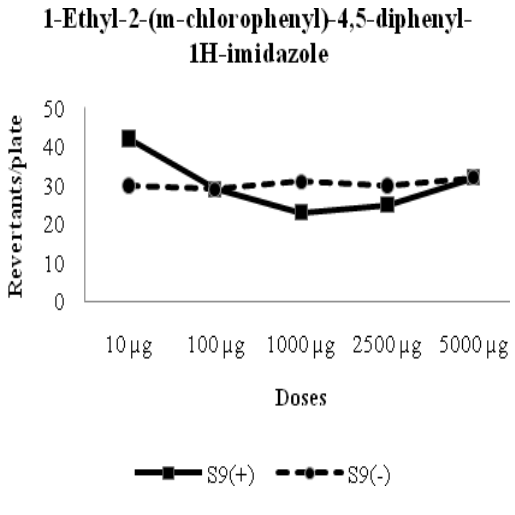
The Ames assay was performed according to the plate incorporation method of Ames and Maron (1983). The *S. typhimurium* TA98 strain is used for the determination of frame shift mutations, the *S. typhimurium* TA100 strain is used for the determination of base pair substitutions [26]. The plate incorporation method was conducted in two parts, with and without the S9 fraction. In the part without the S9 fraction, 0.1 ml of over-night (14-16 h, $1.2x10^9$ cfu/ml) bacteria culture, 0.1 ml of test substance, and 0.2 ml of histidine-biotin were added to the top agar, poured into glucose agar plates and spread. In the experiments with S9, 0.5 ml of S9 fraction was added to the prepared mixture. Each dose of the compound was tested three times and all experiments were replicated twice. Plates were incubated at 37°C for 48-72 hours. The colony numbers of three distinct plates were counted and the means of the colony numbers and standard deviations were calculated. The means of these colony numbers (with the compound) should be at least two fold higher than spontaneous revertant colony numbers (without the compound) in order to consider the tested compound as a mutagen.

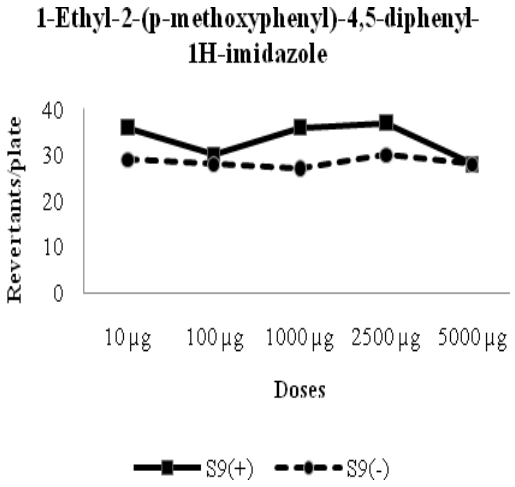
3. RESULTS

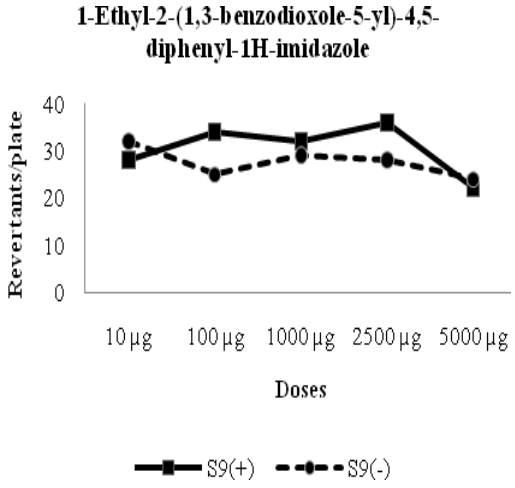
The results of the test chemicals with and without S9 fraction in the *S. typhimurium* TA98 and TA100 strains are given in Figures 2 and 3. Spontaneous revertant colony numbers were found to be 41±6 (with S9), and 24±3 (without S9) for *S. typhimurium* TA98. For *S. typhimurium* TA100, it was found to be 181±20 with S9 and 119±21 without S9. Revertant colony numbers of *S. typhimurium* TA98 and TA100 strains that were formed by positive controls with (+) and without (-) S9 are given in Table 1.

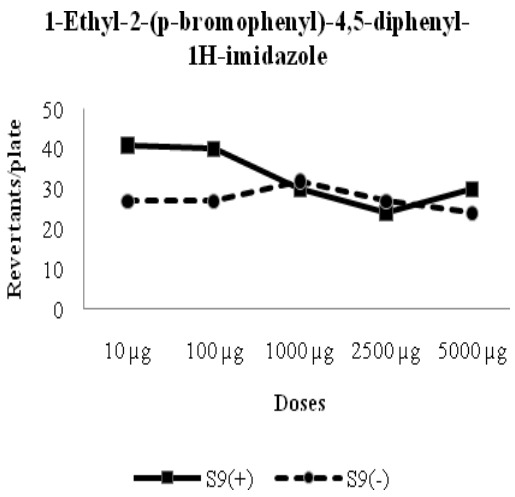












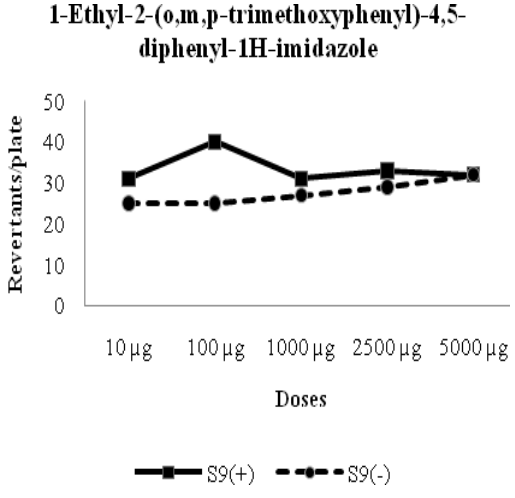
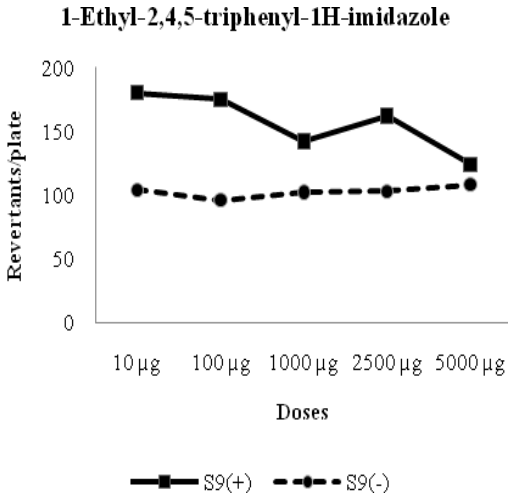
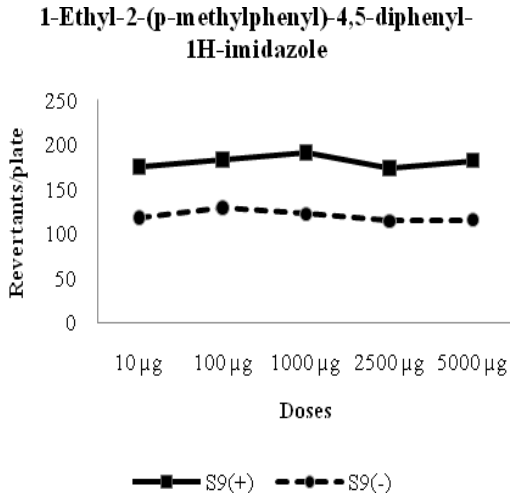
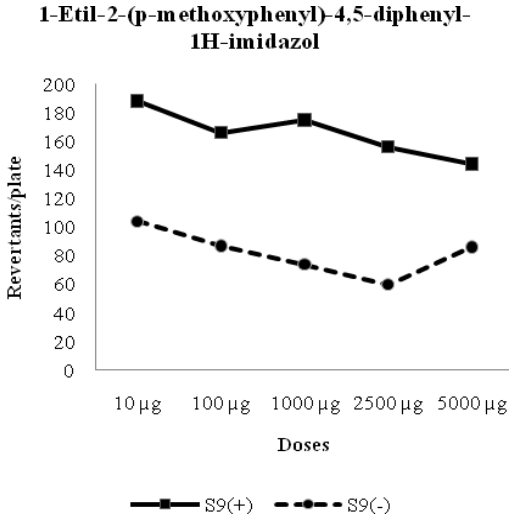
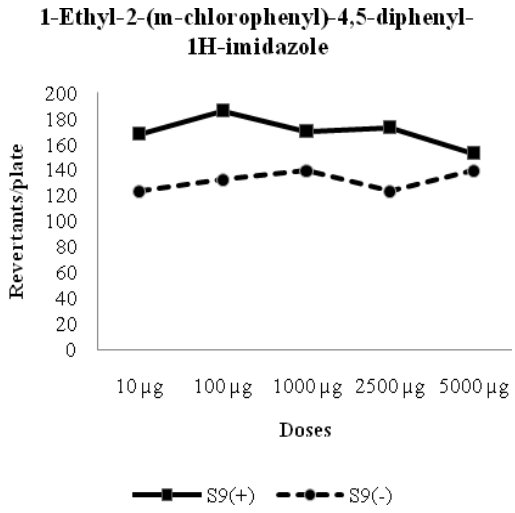


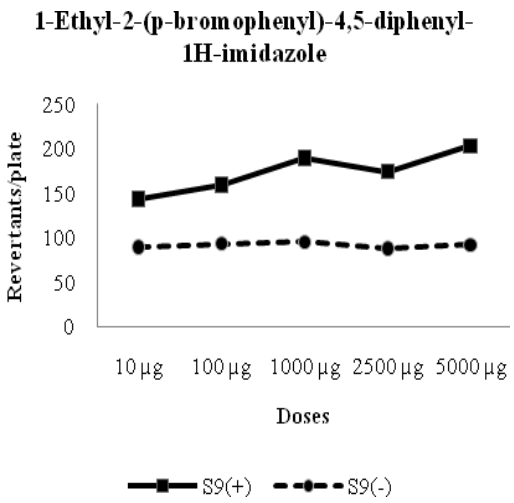
Figure 2. Revertant colony numbers of test compounds in *S. typhimurium* TA98 strain with and without S9.

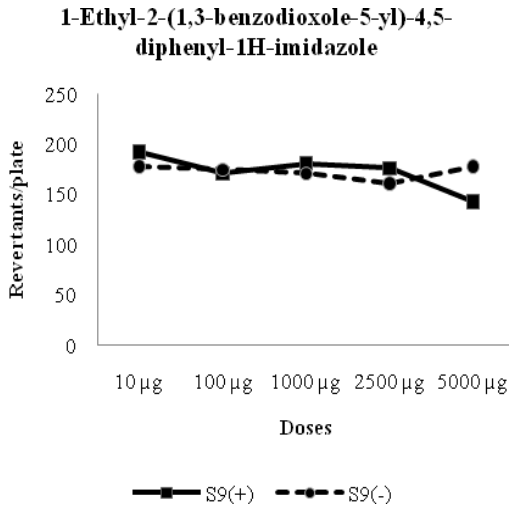

1-Ethyl-2,4,5-triphenyl-1H-imidazole
Revertants/plate
200
150
100
50
0
10 µg
100 µg
1000 µg
2500 µg
5000 µg
Doses
S9(+)
S9(-)


1-Ethyl-2-(p-methylphenyl)-4,5-diphenyl-1H-imidazole
Revertants/plate
250
200
150
100
50
0
10 µg
100 µg
1000 µg
2500 µg
5000 µg
Doses
S9(+)
S9(-)


1-Etil-2-(p-methoxyphenyl)-4,5-diphenyl-1H-imidazol
Revertants/plate
200
180
160
140
120
100
80
60
40
20
0
10 µg
100 µg
1000 µg
2500 µg
5000 µg
Doses
S9(+)
S9(-)


1-Ethyl-2-(m-chlorophenyl)-4,5-diphenyl-1H-imidazole
Revertants/plate
200
180
160
140
120
100
80
60
40
20
0
10 µg
100 µg
1000 µg
2500 µg
5000 µg
Doses
S9(+)
S9(-)


1-Ethyl-2-(p-bromophenyl)-4,5-diphenyl-1H-imidazole
Revertants/plate
250
200
150
100
50
0
10 µg
100 µg
1000 µg
2500 µg
5000 µg
Doses
S9(+)
S9(-)


1-Ethyl-2-(1,3-benzodioxole-5-yl)-4,5-diphenyl-1H-imidazole
Revertants/plate
250
200
150
100
50
0
10 µg
100 µg
1000 µg
2500 µg
5000 µg
Doses
S9(+)
S9(-)

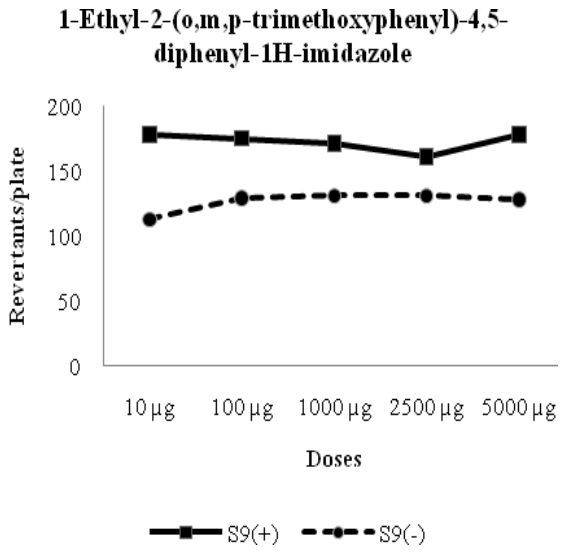


Figure 3. Revertant colony numbers of test compounds in *S. typhimurium* TA100 strain with and without S9.

Table 1. Revertant colony numbers of *S. typhimurium* TA98 and TA100 strains were formed by positive controls with (+) and without (-) S9.

Chemicals (µg/plate)	***S. typhimurium* TA98 revertant colony number**		***S. typhimurium* TA100 revertant colony number**	
	S9(-)	S9(+)	S9(-)	S9(+)
2-aminofluoren(10)	-	671±100	-	1704±156
Sodium azide (1.5)	-	-	400±150	-

4.DISCUSSION

In the study, mutagenicity of 1-Ethyl-2,4,5-triphenyl-1H-imidazole and six derivatives were investigated using *S.typhimurium* TA98 and TA100 mutant strains. None of the tested compounds was detected as mutagenic in the strains with and without the S9 fraction. In other words, the number of colonies reversed from auxotroph to prototroph did not reach two fold the spontaneous revertant colony numbers. In this respect, it could be stated that these compounds do not have direct and indirect mutagenic effects.

Several studies have been conducted by other researchers who also indicated that some imidazole compounds and metabolites are not mutagenic in Ames assays. Forster et al. (1992) reported non-mutagenic effects of hydantoic acid, hydantoin and N-acetyl-imidazole by Ames assay [27]. Additionally, in the study by Voogd et al. (1979), imidazole and metabolites were reported as not to be mutagenic [28]. In another study, it was revealed that imidazole has no mutagenic and bactericidal effects on TA1535, TA100, TA1537 and TA98 strains in Ames assays with metabolic activation [29]. In the study of Pérez-Rivera et al. (2009), eight distinct imidazole derivatives named as butoconazole, econazole, miconazole, oxiconazole, sulconazole, tioconazole, clotrimazole and ketoconazole also gave negative results in bacterial mutation assays [19].

On the other hand, some imidazole derivatives are known to have mutagenicity. Josephy et al. (1996) reported that 2-amino-6-mEthyldipyrido [1,2-a:3,2-d] imidazole and about 20 heterocyclic amines derived from cooked food were strong mutagens [30]. Additionally, 4-nitro imidazole derivatives were synthesized as novel antimycotic and it was evaluated that these compounds have week mutagenic effects on TA98 and TA100 strains with/without metabolic activation [21].

In the literature, it was reported that the positions of side groups of imidazole ring have an important influence on a compound's structure and its mutagenic activities. It was pointed out that most of the imidazole derivatives caused fewer mutagenic responses on the TA100 strain than on the TA98. Hrelia et al. (1997) showed that the presence of methyl and benzyl groups in imidazole ring and substituents in N1 and N3 positions are deterministic for mutagenicity [21]. According to Aydogan and Kutlu (2007), the 1-Ethyl-4,5-diphenyl-1H-imidazole compound did not cause mutagenic effects in the presence of S9 towards the *S. tyhimurium* TA100 strain. On the contrary, under the same conditions this compound carrying a methyl group on 2. position of the imidazole ring was found to be mutagenic [31]. Another study on imidazole derivatives of the *S. tyhimurium* TA98 strain showed that the 2-(2-hydroxyphenyl)-4,5-bis-(4-methylphenyl) imidazole compound carrying a

methyl group on the C4 position exhibited strong mutagenicity, while the compound with a methoxy group on the same position showed poor mutagenic activity [17].

In conclusion, linking number, position and order of substituents on an imidazole ring is important for the mutagenic activities of the new compounds. Therefore, before their use in daily life, the determination of the mutagenic potential of new imidazole derivatives is essential for human health and the environment.

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ACKNOWLEDGEMENTS

We are especially grateful to Prof. Dr. İlhan IŞIKDAĞ and Assoc. Prof. Dr. Asiye MERİÇ (Anadolu University, Faculty of Pharmacy, Eskişehir, Turkey) for gifting the test compounds. This study was financed by the Eskişehir Osmangazi University Scientific Project Committee as Project number 1996 19-043.

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