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Mutagenicity Studies of some Substituted Benzylideneaniline Derivatives

F. Susuz Alanyali, Ö. Artagan and S. Yüksel
Department of Biology, Faculty of Sciences, Anadolu University, 26470, Eskişehir, Turkey

Abstract: The aim of this study was to evaluate the mutagenic potential of newly synthesized eight Benzylideneaniline derivatives by using the *Salmonella* mutagenicity test, using two strains of *Salmonella typhimurium* carrying frameshift (TA 98) and base-pair substitution (TA 100) mutations. Strains were used in plate incorporation method in the absence and presence of metabolic activation. In a general manner, 4-chloro, 4-methoxy, 4-nitro, 4-methyl, 2 methoxy, 3-nitro derivatives were found to be mutagenic in TA 100 in the absence of metabolic activation. 4-hydroxy benzylidenaniline and 4-bromo benzylidenaniline exhibited direct mutagenicity in TA 98 in the absence of metabolic activation. In the presence of S9 (metabolic activation) mix, 4-methoxy benzylidenaniline, 4-nitro benzylidenaniline, 2-methoxy benzylidenaniline, 3-nitro benzylidenaniline compounds gave mutagenic results in TA 98 and 4-methoxy benzylidenaniline, 2-methoxy benzylidenaniline, 3-nitro benzylidenaniline compounds in TA 100. Mutagenicity of 4 compounds (4-chloro, 4-nitro, 4-methyl, 4-hydroxy) completely disappeared in TA 100 strain by the use of S9 (metabolic activation) mix. Compounds with nitro and methoxy groups exhibited greater mutagenicity in both strains in the presence and absence of metabolic activation.

Key words: Mutagenicity, benzylideneaniline, *Salmonella*, ames test/microsome, metabolic activation

INTRODUCTION

Nowadays, proportionally with the advances in technology, human beings are affected by various chemical materials and additives, particularly drugs which have been used widespread (Jagetia *et al*., 2001). Chemicals, both manufactured and natural play an important role in the cause of human cancer. Determination of carcinogenic and mutagenic compounds and evaluation of their effects are of great significance in order to protect human population from such substances (Jagetia *et al*., 2001; Maron and Ames, 1983; Wyszyn and Liro, 1991; Claxton and George, 2007). The *Salmonella* mutagenicity test (Ames test) is widely used short-term bacterial reversion mutation assay firstly described by Dr. Bruce Ames (Ames *et al*., 1975; Mortelmans and Zeiger, 2000). In this test histidine dependent *Salmonella* strains carrying different mutations in varius genes in the histidine operon (Maron and Ames, 1983; Waters *et al*., 1990; Mortelmans and Zeiger, 2000). It is used world-wide as an initial screen to determine the mutagenic potential of new chemicals and also used for submission of data to regulatory agencies for registration and acceptance of many chemicals including drugs. The identification of chemicals capacity of inducing mutations is an important procedure in safety assessment. The continuing popularity of *Salmonella* mutagenicity test depends on the ability of prescreening large number of chemicals with high sensitivity (Josephy *et al*., 1997; Mamber *et al*., 1993; Kaplan *et al*., 2004). Chemicals that can induce mutations can potentially damage the germ line leading to fertility problems and cancer (Mortelmans and Zeiger, 2000; Kutlu *et al*., 2007; Claxton and George, 2007). Mutations can cause gene (point) mutations, single base or a few bases are inserted or deleted, as large deletions or rearrangements of DNA, as chromosome breaks or rearrangements, gain or loss of whole chromosomes (Mortelmans and Zeiger, 2000). Ames test has a high predictive value for carcinogenicity (Aydogan and Kutlu, 2007; Benkli *et al*., 2009).

Benzylideneaniline derivatives are examples of simple Schiff bases. Schiff bases are a class of important compounds in the medicinal and pharmaceutical field. They show several biological activities including antibacterial, antifungal, herbicidal, antiviral, antioxidant, antiinflammatory activities. Furthermore, Schiff bases are utilized as starting material in the synthesis of industrial and biological compounds such as b-lactams and their metal complexes have an important pharmacological effect because of antineoplastic, antimalarial, antifungal, antibacterial and anticancer features (Chung *et al*., 2000).

Corresponding Author: F. Susuz Alanyali, Department of Biology, Faculty of Sciences, Anadolu University, 26470, Eskişehir, Turkey Tel: 0 90 222 335 0580/4726 Fax: +90-222-3204910

The present study aims to investigate the potential mutagenicity of eight newly synthesized benzylideneaniline derivatives in *Salmonella* strains TA 98 and TA 100. In this connection plate incorporation assay was performed according to Maron and Ames (1983) and Mortelmans and Zeiger (2000). The results were discussed in view of estimating the relationships between benzylideneaniline, its substituents and mutagenicity potentials of the derivatives.

MATERIALS AND METHODS

This research was studied between 2004-2005 at Biology Department laboratuar.

Chemicals: Dimethyl sulfoxide (DMSO) and 4-nitro-o-phenylenediamine (NPD) were purchased from Aldrich. D-glucose-6 phosphate disodium salt, L-histidine. HCI, D-biotin, 2-aminofluorene and ampicillin trihydrate were from Sigma. Sodium azide (SAZ) was from Merc. Nutrient broth and bacto agar were purchased from Oxoid.

Samples: Benzylideneaniline derivatives were kindly provided from Dr. Ilhan Isikdag, Anadolu University, Faculty of Pharmacy, Eskisehir, Turkey.

Bacterial strains: Histidine dependent mutant strains of *Salmonella typhimurium* TA 100 and TA 98 were kindly provided from Dr. Bruce.N. Ames, University of California, Berkeley, USA and strains kept as described by Maron and Ames (Maron and Ames, 1983). Tester strains were checked for genetic analysis; histidine dependence, rfa marker, UV sensitivity (uvr B mutation), presence of plasmid pKM101 and spontaneous mutant colony frequency.

Mutagenicity assay: The concentrations were selected on the basis of toxicity. Doses were evaluated between the highest nontoxic dose and the lowest toxic dose in this toxicity assay. Toxicity was apparent either as a reduction in the number of his revertants, or as an alteration in the background lawn.

Samonella strains of TA 98 and TA 100 were used with and without metabolic activation by the standard plate incorporation method (Maron and Ames, 1983). Doses of benzylideneaniline derivatives were determined by a preliminary cytoxicity test. In the standard plate incorporation method, 100 μL of the fresh overnight culture of the tester strain (approximately 1×10^9 bacteria/mL) and 100 μL of the test chemical were added into the 2 mL of soft agar in 45°C. Tubes were vortexed a few seconds then poured onto a minimal glucose agar plate. Metabolic activation was provided by adding 0.5 mL of S9 mix to the reaction medium. The plates were incubated at 37°C for 48 h DMSO was used as the solvent control. 4-nitro-o-phenylenediamine (NPD) (20 μg per plate) and sodium azide (AZS) (1, 5 μg per plate) were used as positive controls for TA 98 and TA 100 strains, respectively without S9 fraction. 2-Aminofluorene (2AF) (10 μg per plate) was used for both strains in the presence of S9 mix. All plates were performed in triplicate (Maron and Ames, 1983).

Statistical analysis: A test sample was classified as mutagenic if it produced a dose-response curve and if it gives a significant response in at least one concentration more than the control incidence (Maron and Ames, 1983). The mutagenicity test results were evaluated using Student's t-test. The results were considered to be statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Salmonella typhimurium strains TA 98 and TA 100 displayed different mutagenic sensitivities to compounds in this study.

4 hydroxy benzylidenaniline gave mutagenic response with the doses of 10 and 100 μg plt^{-1} in TA98 strain without metabolic activation (Fig. 1). When S9 mixture was added in 4 hydroxy, mutagenicity disappeared in TA98 (Fig. 2).

4 chloro benzylidenaniline gave mutagenic response only TA100 strain (0.1, 1, 10 μg plt^{-1}) (Fig. 1). 4 chloro benzylidenaniline was not mutagenic with S9 (metabolic activation) (Fig. 2).

4-methoxy benzylidenaniline did not exhibit any mutagenicity on *Salmonella typhimurium* strain TA98 without metabolic activation (S9) (Fig. 1). On the other hand it gave mutagenic response on TA100 strain at doses of 0.01, 0.1, 1, 100 μg plt^{-1} without S9 (Fig. 1). 4-methoxy mutagenically effective on TA98 at the doses of 0.01, 0.1, 10, 100 μg plt^{-1} in the presence of S9 mixture. In presence of S9 4-methoxy gaved mutagenic response at the doses of 10, 100 μg plt^{-1}. Four nitro benzylidenaniline did not gave any mutagenic response on TA98 without S9 (Fig. 1) and on TA100 with S9. TA 98 strain with metabolic activation (S9) showed mutagenicity only 100 μg plt^{-1} dose. 4-nitro, at the doses of 1, 10, μg plt^{-1} on TA 100 strain of *Salmonella* was mutagenic in the presence metabolic activation (Fig. 2).

4-methyl benzylidenaniline gaved mutagenic response only 1, 10, 100 μg plt^{-1} doses on TA 100 strain without metabolic activation (Fig. 1). 4-bromo benzylidenaniline did not exhibit any mutagenicity in the presence or absence of S9 mix on TA100 strain of *Salmonella* (Fig. 1-2).

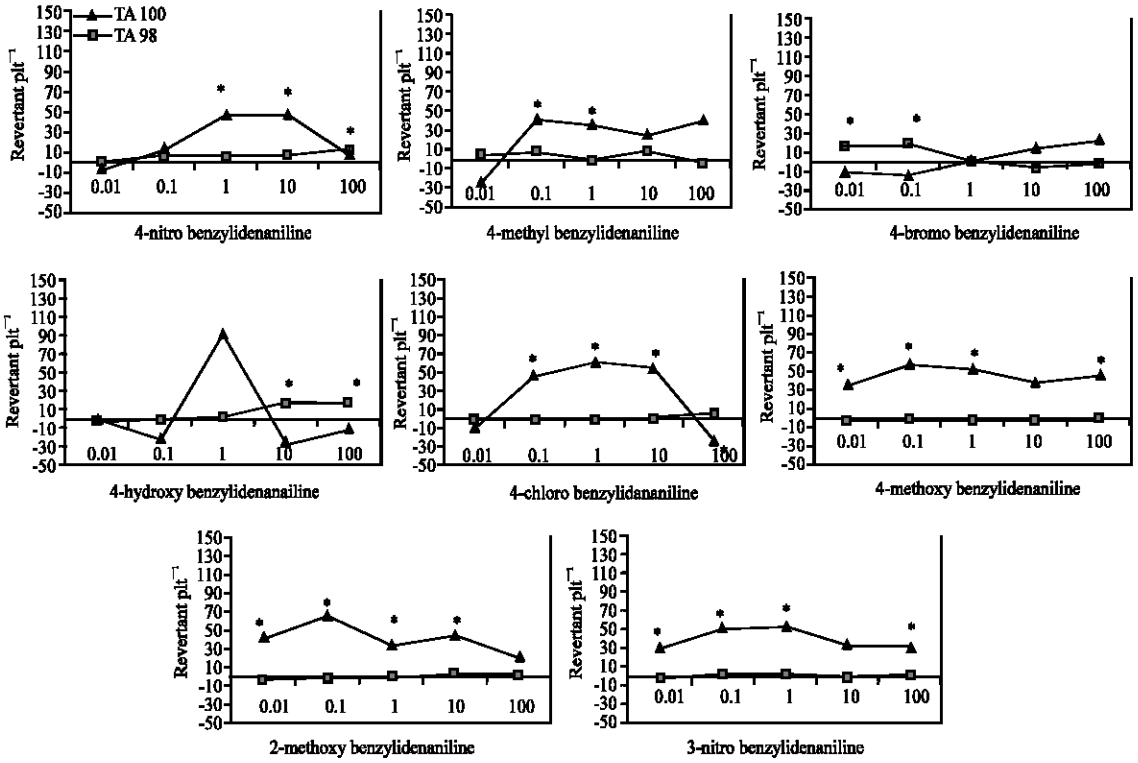

Fig. 1: Dose-response (0.01, 0.1, 1, 10, 100 μg plat^{-1}) curves of the revertants induced by the tested compounds in the absence of metabolic activation. The values of solvent control (DMSO) 21.3±6.8 (TA 98), 117.6±.6 (TA 100) were subtracted. Positive controls: TA 98: NPD (20 μg plat^{-1}) 1516±37.4 TA 100: AZS (1.5 μg plat^{-1}) 714±82.3. *p≤0.05

4-bromo showed mutagenicity at the doses of 0.01 and 0.1 without S9 mix on TA98 (Fig. 1). 1 μg plt^{-1} dose showed mutagenicity in the presence of S9 (Fig. 2).

2-methoxy benzylidenaniline on TA98 *Salmonella* strain was not found mutagenic without S9 mix. On the other hand all doses of 2-methoxy gave mutagenic response on TA98 in the presence of metabolic activation (Fig. 2). 0.01, 0.1, 1, 10 μg plt^{-1} doses of 2-methoxy on TA100 without S9 mix were found mutagenic (Fig. 1).

None of the doses of 3 nitro benzylidenaniline without S9 mix showed any mutagenicity on TA98 strain (Fig. 1).

In a general manner, compounds or their metabolites tested were found mutagenic at least for one of the strains. In the absence of S9, compounds tested have had a greater mutagenic effect causing base-pair substitution mutations in TA100 strain (Fig. 1). Six of the eight compounds except 4 bromo and 4 hydroxy were found to be mutagenic for TA 100 (Fig. 1). Mutagenicity of 5 (4 nitro, 4 methyl, 4 bromo, 4 chloro, 4 hydroxy) of these compounds disappeared by the addition of S9 mix to the reaction medium (Fig. 2). In the presence of S9, 4 (4 nitro, 3 nitro, 4 methoxy, 2 methoxy) compounds were causing frame-shift mutations in TA98 (Fig. 1) and 3 of them except 4 nitro, obtained this mutagenic property by the addition of S9 mix (Fig. 2). Compounds carrying a nitro group in position 3 and methoxy group in positions 2 and 4 were considerably mutagenic for both strains of *Salmonella* in the presence of S9 (Fig. 2). Some substituents in different positions can cause different interactions with DNA. (Aydogan and Kutlu (2007), Mortelmans and Zeiger (2000) and Özkay *et al*. (2006) reported that the addition of methoxy group to benzidine molecule increased the mutagenicity. They also indicated that introduction of a nitro group to a benzene ring would greatly increased its mutagenicity. These reports showing the similar methoxy and nitro bound groups increased mutagenicity supporting our results that 4-methoxy, 2-methoxy, 3 nitro bound derivatives in Fig. 1 and 2 showed greater mutagenicity than the others.

The identification of substances capable of inducing mutations is an important procedure in safety assessment. Chemicals that can induce mutations can potentially damage the germ line leading to fertility

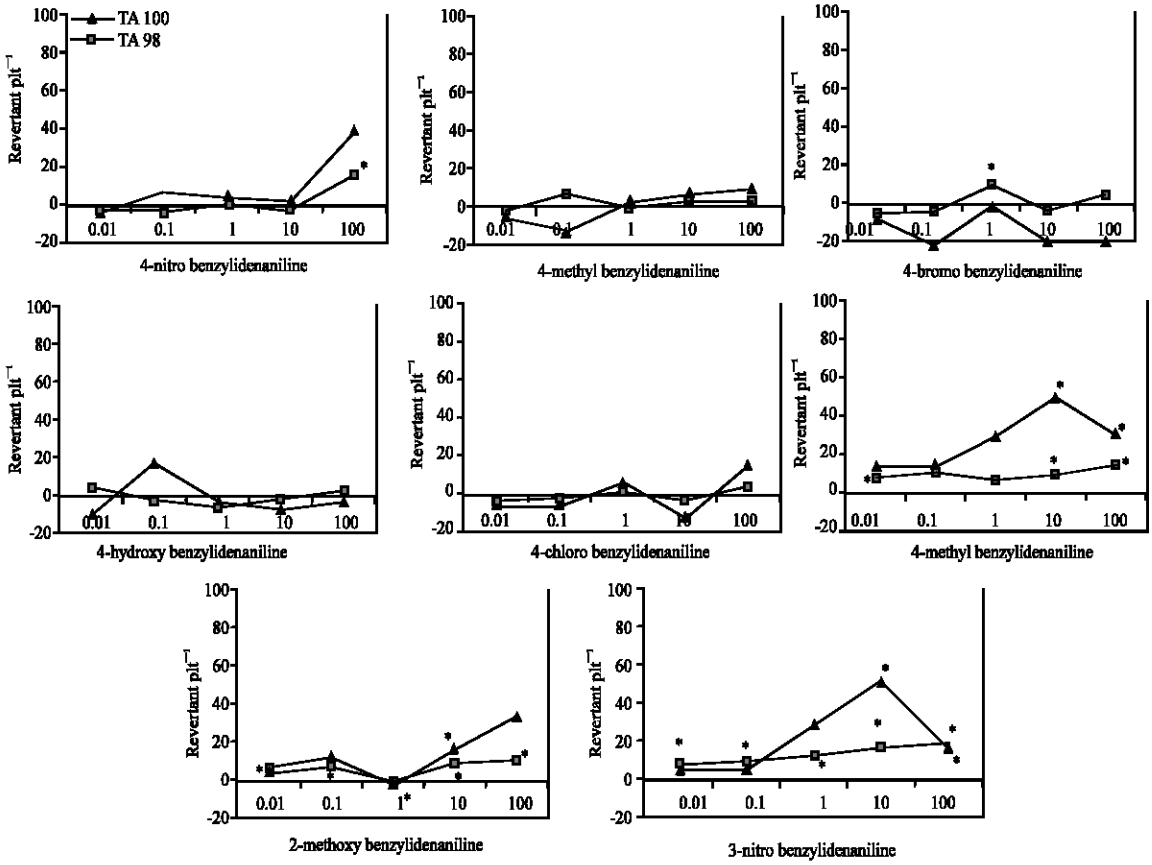

Fig. 2: Dose-response (0.01, 0.1, 1, 10, 100 μg plat^{-1}) curves of the the revertants induced by the tested compounds in the presence of metabolic activation. The values of solvent control (DMSO) 24±3.6 (TA 98), 94±5.6 (TA 100) were subtracted. Positive controls: TA 98: 2 AF (10 μg plat^{-1}) 912±31.5 TA 100: 2 AF (10 μg plat^{-1}) 845±41.6. *p≤0.05

problems (Mortelmans and Zeiger, 2000; Kutlu *et al*., 2007). Although mutagenicity potentials can be evaluated with high sensitivity by the *Salmonella* mutagenicity test, further structure-activity relationships studies are still necessary to associate the chemical properties of the tested compounds and the interactions with DNA.

CONCLUSION

In the presence of rat liver microsomal enzymes (S9 mixture), the mutagenicity was disappeared 4 nitro, 4 methyl, 4 bromo, 4 hydroxy, 4 chloro (except for the highest dose of 4 nitro and 1 μg plt^{-1} dose of 4 bromobenzylidenaniline). Four methoxy, 2 methoxy, 3 nitro bound benzylidenaniline derivatives saved mutagenic response with the addition of S9 mixture.

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