Mutagenicity of Bisbenzimidazole Derivatives

Filiz Susuz Alanyalı[a,*], Merve Arıcı[a], Öge Artagan[a], İlhan Işıkdağ[b], and Yusuf Özkay[b]

[a] Department of Biology, Faculty of Science, Anadolu University, 26470 Eskişehir, Turkey. Fax: +90-222-3204910. E-mail: fsusuz@anadolu.edu.tr
[b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey

* Author for correspondence and reprint requests

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The mutagenicities of 2,2'-(di-3-hydroxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, 2,2'-(di-4-hydroxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, 2,2'-(di-3-methoxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, 2,2'-bis-(4-nitrophenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, 2,2'-bis-(3-nitrophenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, 2,2'-bis-(4-methylphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, 2,2'-(di-4-methoxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, and 2,2'-bis-(3-methylphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole were studied *in vitro* using two strains of *Salmonella typhimurium* with frameshift mutation (TA98) and base-pair substitution mutation (TA100) as the plate incorporation assay in the absence of metabolic activation. These compounds are currently used to treat cancer. 4-Nitrophenyl and 3-nitrophenyl compounds were found to be mutagenic on both strains of *Salmonella*. A clear mutagenic response was seen in nitro-bound derivatives. The mutagenic response in *Salmonella* test strains (TA98, TA100) and structures of molecules suggest that nitro-bound molecules could be mutagenic.

Key words: Mutagenicity, *Salmonella*, Bisbenzimidazoles

Introduction

In clinical use several chemotherapeutic anticancer agents inhibit DNA replication and transcription by the help of nuclear DNA topoisomerases. These enzymes bind temporary one or both DNA strands. Several benzimidazoles are inhibitors of topoisomerases and linked to DNA (Kim *et al.*, 1996). These kind of compounds have an importance in the treatment of AIDS (Neidle *et al.*, 1997; Mann *et al.*, 2001) and show cytotoxic activity against tumour cell lines and positive experimental antitumour activity (Boykin *et al.*, 1995; Francesconi *et al.*, 1999; Mann *et al.*, 2001). The use of chemotherapeutic agents for the prevention of clinical illnesses has highlighted the importance of mutagenicity.

Material and Methods

Chemicals and test strains

Nutrient broth and nutrient agar were obtained from Oxoid, sodium azide (SAZ) was purchased from Merck, D-biotin, L-histidine were from Fluka, ampicillin trihydrate, D-glucose-6 phosphate and dimethyl sulfoxide (DMSO) were purchased from Sigma, 4-nitro-*o*-phenylenediamine (NPD) was purchased from Aldrich. *Salmonella typhimurium* strains TA98 (frameshift mutation) and TA100 (base-pair substitution mutation) were supplied from Professor B. N. Ames (University of California, Berkeley, CA, USA). Genotype controls of test strains were confirmed according to Maron and Ames (1983).

Salmonella mutagenicity testing

The standard plate incorporation assay was performed according to Maron and Ames (1983), by adding 0.1 mL of the fresh overnight culture (approx. 10^8 bacteria) of the test strains, 0.1 mL of different concentrations of test compounds and 0.2 mL of histidine-biotin solution to 2 mL of top agar tubes. Top agar tubes were shaken well and poured onto minimal glucose agar plates. After 48 h of incubation at 37 °C revertant colonies were counted. 20 μg/plate NPD and 1.5 μg/plate SAZ were used for both test strains as a positive control. DMSO (spectrophotometric grade) was used as the solvent and the solvent control (100 μL/plate). All plates were done in triplicate.

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Determination of the mutagenicity

Chemicals were thought to be mutagenic when the number of revertant colonies in the test plate doubled the number of revertant colonies in the spontaneous control plate. Mutagenicities of the bisbenzimidazole derivatives were investigated with TA100 and TA98 by the plate incorporation method without addition of metabolic activation (S9) mixture.

Results and Discussion

2,2'-Bis-(4-methylyphenyl)-1*H*,1*H*'-[5,5']-bis-benzimidazole (Fig. 1), in TA98 and also TA100

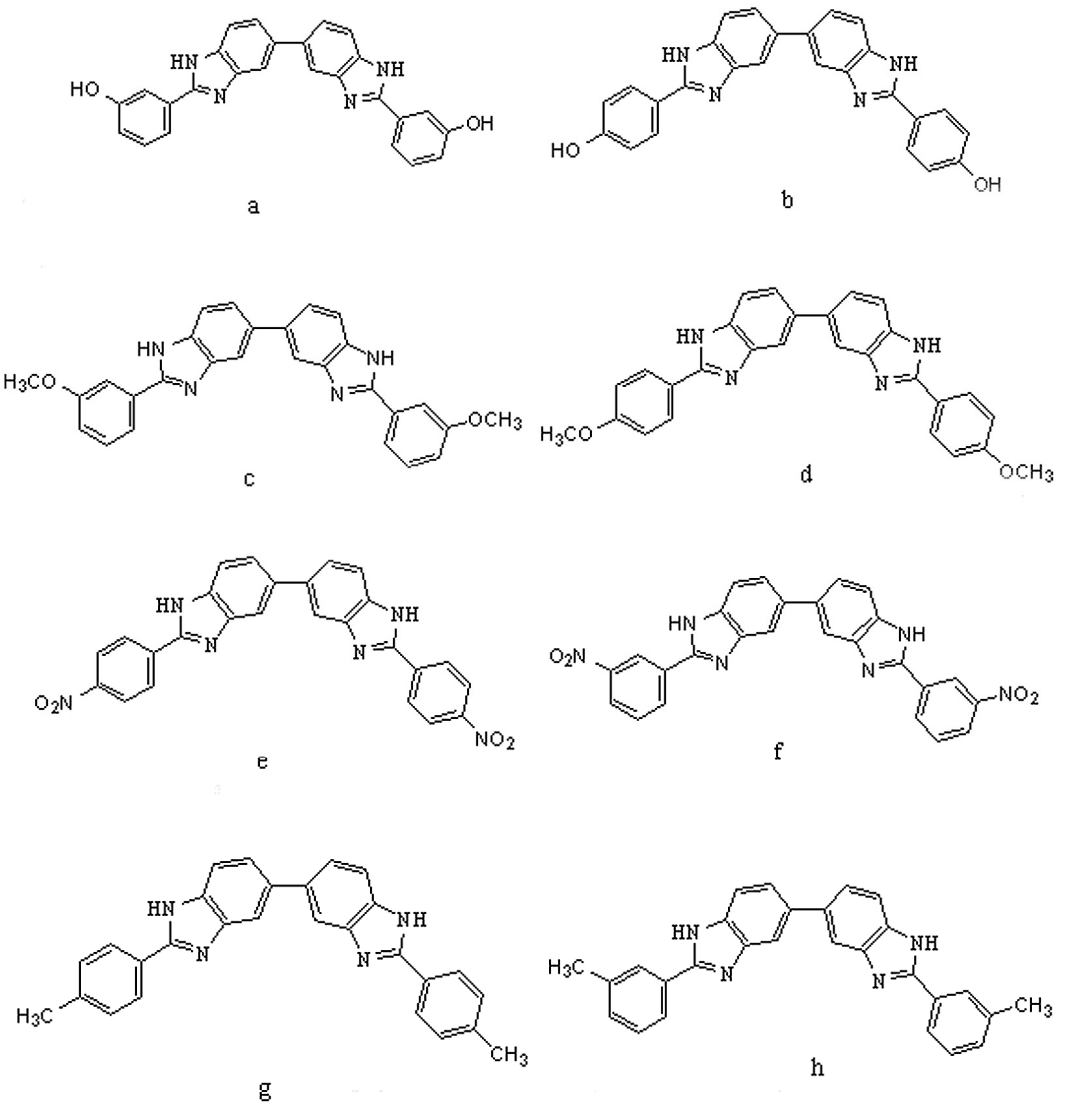


Fig. 1. Chemical structure of (a) 2,2'-(di-3-hydroxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (b) 2,2'-(di-4-hydroxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (c) 2,2'-(di-3-methoxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (d) 2,2'-(di-4-methoxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (e) 2,2'-bis-(4-nitrophenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (f) 2,2'-bis-(3-nitrophenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (g) 2,2'-bis-(4-methylphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (h) 2,2'-bis-(3-methylphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole.

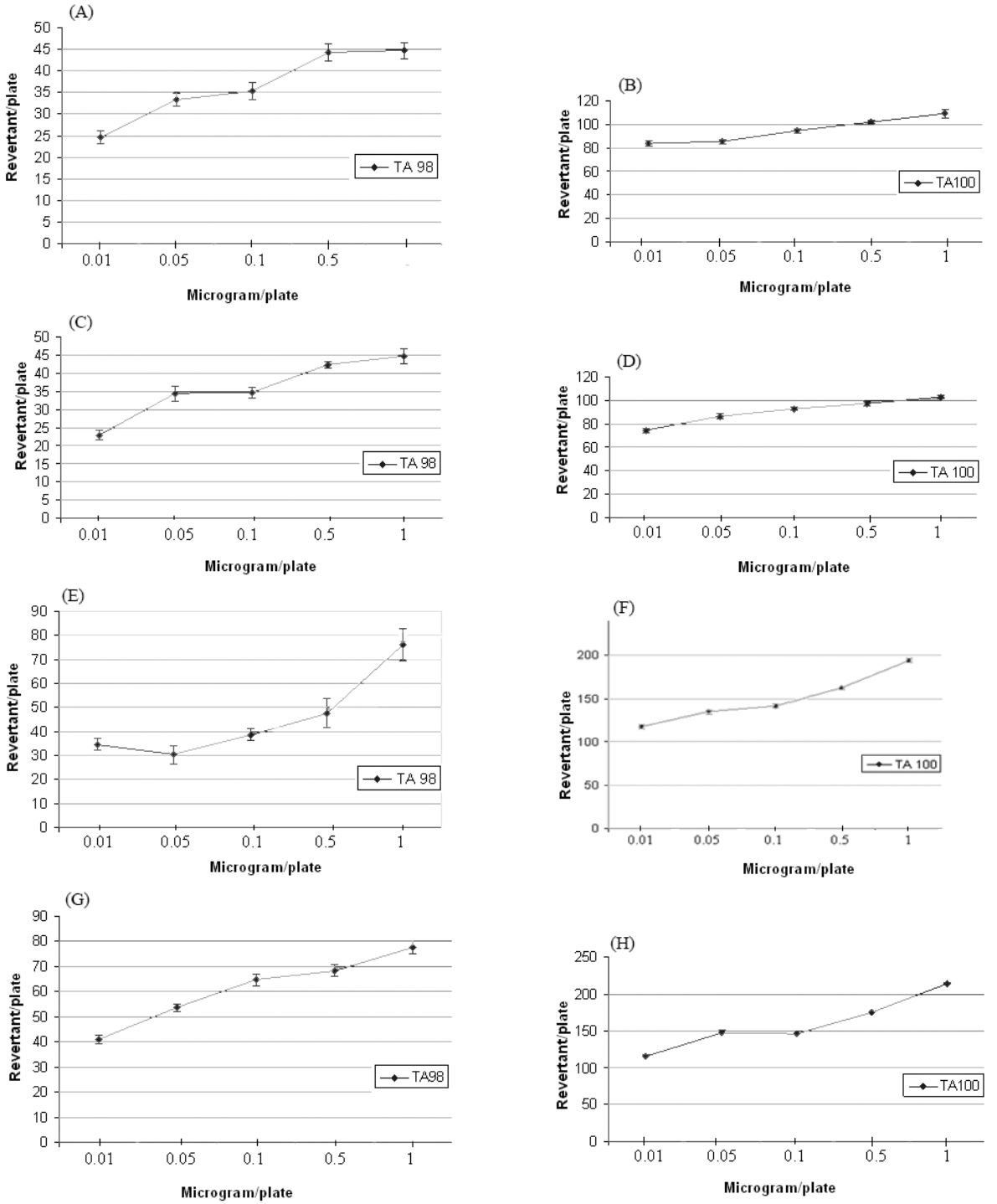
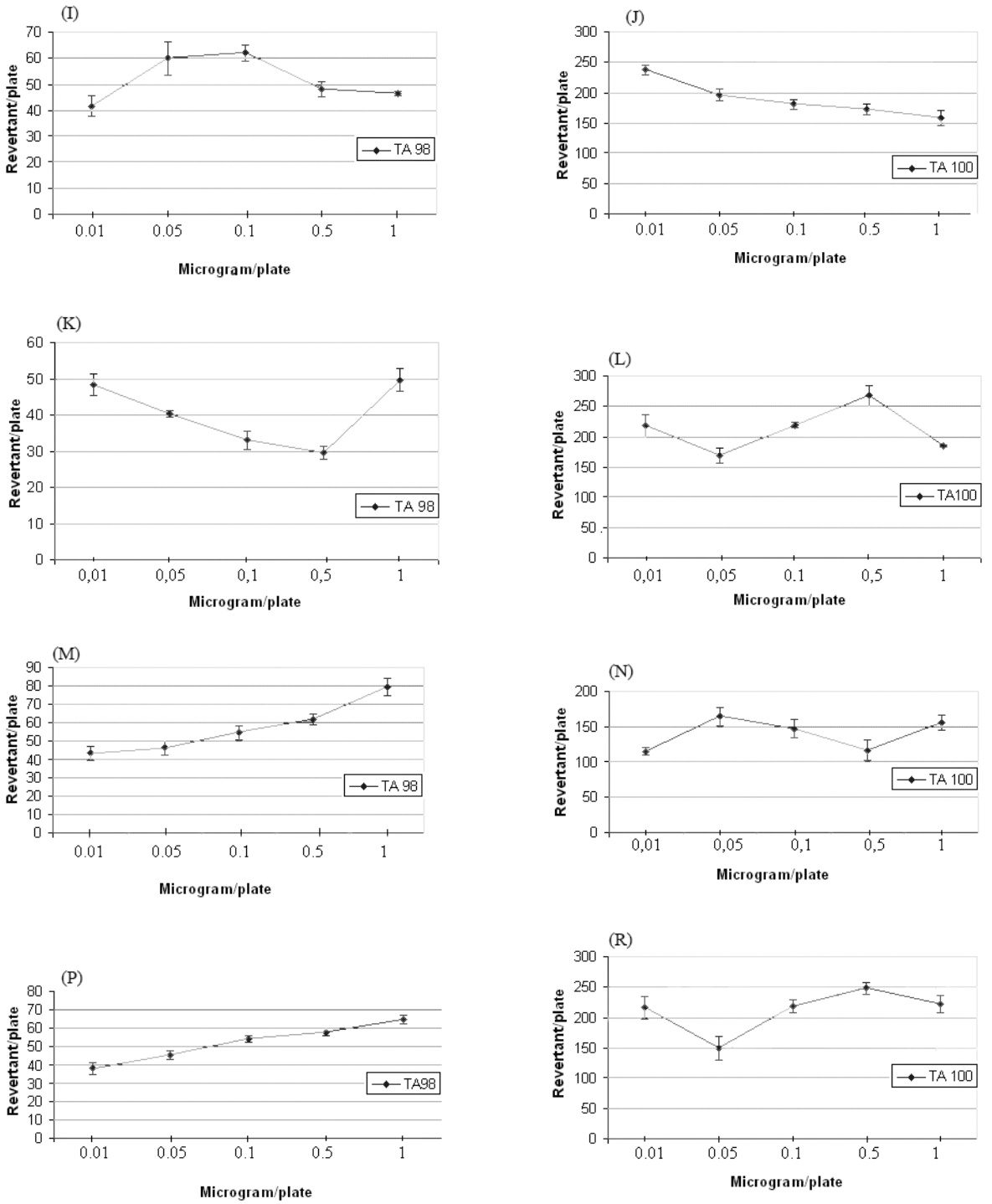


Fig. 2. Mutagenicity of (A, B) 2,2'-bis-(4-methylphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (C, D) 2,2'-(di-3-hydroxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (E, F) 2,2'-(di-3-methoxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (G, H) 2,2'-(di-4-methoxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (I, J) 2,2'-(di-4-hydroxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (K, L), 2,2'-bis-(3-methylphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole, (M, N) 2,2'-bis-(3-methylphenyl)-



1*H*,1*H*'-[5,5']-bisbenzimidazole, (P, R) 2,2'-bis-(4-nitrophenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole. The number of spontaneous revertants was subtracted. The spontaneous frequencies of his$^+$ revertants (range) were: TA100: 122 – 168 and TA98: 26 – 46. Negative control (solvent): 100 μL DMSO, TA100: 126 and TA98: 38. Positive control: 20 μg/plate 4-nitro-*o*-phenylenediamine (NPD), TA98: 385 – 432; 1.5 μg/plate sodium azide (SAZ), TA100: 532 – 568.

strain, revealed a dose-releated increase. In TA100 strain weak mutagenic activity was observed for both 0.5 and 1 μg/plate doses and none in TA98 (Figs. 2A, B). 2,2'-(Di-3-hydroxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole (Fig. 1) showed a dose-related increase in the number of revertant colonies in both of the two strains, TA98 and TA100, but the number of revertant colonies was not double the number of spontaneous revertant colonies. For this reason there was not any mutagenicity (Figs. 2C, D). Although there was a slight increase in the number of revertant colonies at the doses of 0.01, 0.05, 0.1, 0.5, 1 μg/plate of 2,2'-(di-3-methoxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole in TA98 and TA100 strains, this compound is not mutagenic, since the number of revertant colonies is not more than double of the number of the spontaneous colonies (Figs. 2E, F). The revertant colony number of 2,2'-(di-4-methoxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole dose-related increased from 0.01 to 1 μg/plate in both strains of *Salmonella.* In TA98 strain of *Salmonella* 0.1, 0.5, 1 μg/plate doses revealed mutagenicity. In TA100 strain 0.5, 1 μg/plate doses gave mutagenic response (Figs. 2G, H). 0.1 μg/plate of 2,2'-(di-4-hydroxyphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole revealed maximum revertant colonies in TA98 strain but revertants were not double the spontaneous revertant colonies. In TA100 a dose-related decrease appeared. There was not any mutagenicity observed for the both strains of *Salmonella* (Figs. 2I, J). 1 μg/plate of 2,2'-bis-(3-methylphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole revealed maximum revertant colonies in *Salmonella* strain TA98, but it can be seen that there was no significant increase of revertants due to the treatment with the chemical. 0.5 μg/plate of 2,2'-bis-(3-methylphenyl)-1*H*,1*H*'-[5,5']-bisbenzimidazole showed a mutagenic effect on *Salmonella* strain TA100 (Figs. 2K, L). 2,2'-Bis-(3-nitrophenyl)-1-*H*,1*H*'-[5,5']-bisbenzimidazole showed a dose-releated increase in TA98 the revertant colonies. Although 0.5 μg/plate revealed slight mutagenicity, 1 μg/plate showed mutagenic response on TA98 strain. In TA100 strain, mutagenicity was observed for both 0.05 and 1 μg/plate doses (Figs. 2M, N). At the doses of 0.5 μg/plate and 1 μg/plate 2,2'-bis-(4-nitrophenyl)-1*H*,1*H*'2-[5,5']-bisbenzimidazole was mutagenically effective on TA98. This compound was found mutagenic on TA100 except for 0.05 μg/plate (Figs. 2P, R).

The chemicals were studied in the absence of metabolic activation. Nitro-bound bisbenzimidazole derivatives revealed to be mutagenic on test strains (TA98, TA100). 4-Methoxyphenyl-bound compounds tend to be mutagenic on *Salmonella* strains TA98 and TA100. 3-Methylphenyl-bisbenzimidazole showed a mutagenic effect on *Salmonella* strain TA100. These results are guiding rules in order to maintain a primary mutagenic effect of chemicals. In further researches, experiments should be repeated with S9 (rat liver homogenate) in order to have knowledge about mutagenic activity in eucaryotic organisms.

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