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# EVALUATION OF GENOTOXITY AND MUTAGENICITY OF DL-P-CHLOROPHENYLALANINE, ITS METHYL ESTER AND SOME N-ACYL DERIVATIVES

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DL-p-chlorophenylalanine (PCPA) and its derivatives were evaluated for genotoxic effects using *Escherichia coli* and *Bacillus subtilis* strains lacking various DNA-repair mechanisms in spottest and in suspension test. The mutagenic activity of studied compounds was determined by the Ames test. Reverse mutation test was performed with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 without S9 mix. 0.02 M nitrosomethylurea (NMU) standard mutagen was used as a positive control. The results showed that the parent nonessential amino acid PCPA had no detectable genotoxic and mutagenic activities in bacteria. The methyl ester of this amino acid and its N-phenylacetyl derivative possessed weak genotoxicity. Meanwhile N-sec-butyloxycarbonyl, N-benzyloxycarbonyl, N-(p-nitrophenylacetyl) and N-(p-nitrophenoxyacetyl) derivatives of DL-p-chlorophenylalanine exhibited appreciable genotoxicity. Among the seven tested compounds only N-benzyloxycarbonyl and N-(p-nitrophenoxyacetyl) derivatives of DL-p-chlorophenylalanine have been found to be mutagenic. Only parent PCPA possessed antimutagenic properties in respect of nitrosomethylurea. The structural modification, which strongly affects genotoxicity and mutagenicity perhaps may be due to steric hydrance of the substituents, causing interference with enzyme and DNA interactions.

*Keywords:* Genotoxicity – mutagenicity – DL-p-clorophenylalanine and its derivatives

## INTRODUCTION

p-Chlorophenylalanine (PCPA, Fenclonine) is the long-lasting inhibitor of serotonin (5-hidroxytryptamine, 5-HT) synthesis. This nonessential amino acid blocks the rate-limiting enzyme tryptophan hydroxylase [13, 14]. PCPA causes significant losses of dopamine, noradrenaline and adrenaline in most brain regions [24]. p-Chlorophenylalanine is a valuable experimental tool for study behaviour [20, 23], memory [10, 25], sleep [8, 15], locomotion [5, 24] processes. PCPA specifically inactivated phenylalanine hydroxylase in hepatoma cells in culture [21, 22]. The antineoplastic activity of p-chlorophenylalanine and some its derivatives was shown in experimental models [1, 7, 11, 27, 29, 30]. In clinical investigation PCPA (Fenclonine) possessed positive effect against carcinoid syndrome [6, 28]. p-Chlorophenylalanine inhibited chemotherapy-induced emesis in cancer patients [3]. Thus, p-chloropheny-

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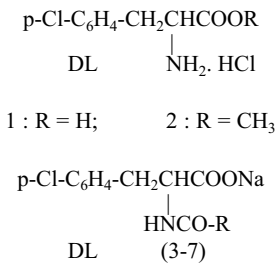
lalanine and some of its derivatives are not only valuable tools in experimental investigations, but PCPA is also promising as a potential drug. In this paper we report the results of the study which was aimed at assessing the genotoxic properties of p-chlorophenylalanine, its methyl ester and some N-acyl derivatives applying bacterial rec assay as a bacterial genetic toxicology test [2, 18] and mutagenic properties determined by Ames test [12, 19].

## MATERIAL AND METHODS

### *Agents*

DL-p-chlorophenylalanine hydrochloride (1), the sodium salts of N-sec-butyloxycarbonyl-DL-p-chlorophenylalanine (3), N-phenylacetyl-DL-p-chlorophenylalanine (4) and N-benzyloxycarbonyl-p-chlorophenylalanine (5) were synthesised according




| Comp. | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|
| R | $CH_3CH_2CHO$ (CH3) | $C_6H_5CH_2$ | $C_6H_5CH_2O$ | p-$NO_2C_6H_4CH_2$ | p-$NO_2C_6H_4OCH_2$ |

*Fig. 1.* Molecular structure of compounds (1–7) used in this study

to the procedure described earlier [29]. DL-p-chlorophenylalanine methyl ester hydrochloride (2) was prepared according to [9]. The sodium salts of N-(p-nitrophenylacetyl)-DL-p-chlorophenylalanine (6) and N-(p-nitrophenoxyacetyl)-DL-p-chlorophenylalanine (7) were synthesised according a procedure described [30]. All tested compounds (1–7) are hydrosoluble. Molecular structures of DL-p-chlorophenylalanine derivatives (1–7) used in the present study are shown in Fig. 1.

## *Bacterial strains*

*Escherichia coli* $K_{12}$C5013 as a wild strain, JC7902 (uvrA$^-$, recA$^-$), AB1886 (*uvrA6*), AB2462 (*recF*), WP6 (*polA1*$^-$), *Bacillus subtilis* BD170 (*rec*$^+$), BD190 (*rec*$^-$), *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 strains were used in this study. All strains were kindly gifted from the Institute of High Molecular Compounds, Moscow, Russia.

## *Growth medium*

Nutrient broth (NB, Difco) with 0.5% NaCl added and LB (Difco) were used for bacterial cells growth. A solid medium contained 2% agar (Difco) [17]. Cultures for tests were grown overnight at 37 °C on a rotary shaker to the definite magnitude of optical density (OD).

## *Genotoxicity assays*

Genotoxic properties of the tested compounds were determined using *Escherichia coli* and *Bacillus subtilis* strains lacking various DNA-repair mechanisms, when compared to the isogenic wild strain by spot test and by growing bacteria in liquid medium [2, 4, 18]. In our study bacteria overnight growing were concentrated at 5000 rpm for 5 min and resuspended in potassium phosphate buffer (0.1 M $Na_2HPO_4 \cdot 7H_2O$, 0.1 M $NaH_2HPO_4 \cdot H_2O$, pH 7,5) to $10^8$ cells/ml when studied compounds were added. Cells were cultured at 37 °C with reciprocal shaking, and the growth was monitored by measuring the $A_{590}$ periodically and number of the cells was compared with cells number in control wild strain suspension. Optical density was measured on photoelectrocolorimetr, using a green filter. All experiments were performed in triplicate.

## *Mutagenic assay*

Mutagenic activity was determined using TA98, TA100, TA1535 and TA1537 *Salmonella typhimurium* strains according to the method described [12, 19]. This assay measures reverse mutations from histidine auxotrophy to prototrophy after exposure to varying dose levels of a test compound. 0.02 M nitrosomethylurea (NMU) standard mutagen was used as positive control. All assay were conducted with 3 replicate plates.

# RESULTS

In order to evaluate the genotoxic activity of the studied compounds we carried out our experiments with *Escherichia coli* and *Bacillus subtilis* strains lacking DNA-repair mechanisms. Inhibition of bacterial growth was evaluated after 18 h at 37 °C.

*Table 1*
Evaluation of genotoxicity of studied compounds with repair deficient system strains after 18 h of incubation by spottest

| Compounds | Strains | | |
|---|---|---|---|
| | *Escherichia coli* | | *Bacillus subtilis* |
| | AB2462 (uvrA6) | WP6 (polA1⁻) | BD190 (rec⁻) |
| 1 | – | – | – |
| 2 | – | – | ± |
| 3 | – | – | + |
| 4 | – | ± | ± |
| 5 | + | – | + |
| 6 | + | ± | ± |
| 7 | + | + | + |

Compounds were used in 2 mM concentration.
(–) – no genotoxic, (±) – weakly genotoxic, (+) – genotoxic

*Table 2*
Evaluation of genotoxicity of studied compounds after 18 h of incubation in the liquid medium

| Compounds | Strains | | | | | |
|---|---|---|---|---|---|---|
| | *Escherichia coli* | | | | *Bacillus subtilis* | |
| | $K_{12}$C5013 | JC7902 | AB2462 | WP6 | BD170 | BD190 |
| | Optical density, % | | | | | |
| 1 | 82 | 80 | 105 | 105 | 144 | 110 |
| 2 | 79 | 84 | 83 | 99 | 103 | 102 |
| 3 | 98 | 10 | 94 | 100 | 10 | 10 |
| 4 | 93 | 80 | 104 | 98 | 59 | 86 |
| 5 | 100 | 82 | 38 | 98 | 12 | 11 |
| 6 | 96 | 82 | 60 | 95 | 107 | 70 |
| 7 | 113 | 87 | 75 | 97 | 69 | 100 |
| Control | 100 | 100 | 100 | 100 | 100 | 100 |

Compounds were used in 2 mM concentration.
$P \leq 0.05$ [16].

Results observed by spottest (Table 1) have shown that compound 1 did not show genotoxic activity in a DNA-repair test in bacteria. Compounds 2, 3 were not genotoxic in experiments with *E. coli uvr* and *E. coli polA⁻* strains, but have shown genotoxic effect in study with *Bac. subtilis rec⁻*. Compounds 4 and 6 have shown weak genotoxic effect against the *polA⁻* strain and *Bacillus subtilis rec⁻* strain. Compound 4 was not genotoxic against *uvr* strain, compound 5 did not show genotoxic effect against *E. coli polA1⁻* strain. However, *E. coli* strain posses *polA1⁻* mutation was less

sensitive to the treatment of tested compounds, except 7. On the other hand, among the repair-deficient strains, the *Bac. subtilis rec⁻* strain was far the most sensitive to the treatment in revealing the direct genotoxicity, especially to 3, 5 and 7 compounds (Table 1). Compound 5 was genotoxic against *Bac. subtilis rec⁻* strain as well as a *E. coli uvr* strain. Among the seven evaluated compounds, only 7 was genotoxic in all studies, and this compound completely inhibited bacterial growth after 18 h. The viability of cells was not restored after subsequent incubation. Our results show that compounds 5–7 exhibited genotoxic activity relative to other compounds. Among seven compounds evaluated only compounds 3 and 5 exhibited the evident genotoxic effect when bacterial strains were grown in liquid medium supplemented with tested compounds (Table 2). The growth of bacteria was completely inhibited and the count of viable cells was not restored during the following incubation. Compounds 4, 6 and 7 exhibited moderate genotoxity. The genotoxic effect of the studied compounds was dose-related. The decrease of concentration from 0.02 mM to 0.002 mM revealed the distinctly expressed normalization of bacterial growth (data not shown).

Other experiments were carried out in order to determine the mutagenic properties of studied compounds. Results presented in Table 3 show that of seven evaluated compounds only compounds 5 and 7 were mutagenic since an increase in the number of revertants was observed with strain TA100 and TA1535, respectively. Furthermore, strain specificity indicated that the molecular mechanisms of tested compounds mutagenicity were affected by base substitution mutagens. Compounds 1–4 and 6 were devoid of mutagenic activity. The absence of mutagenic activity was confirmed in at least 3 experiments. Only compound 1 possessed antimutagenic properties in respect of nitrosomethylurea (Table 3). Antimutagenic effect of 1 compounds was dose-related, increase of the NMU concentration caused the decrease of antimutagenic effect (data not shown).

*Table 3*
Mutagenic activity of the studied compounds on the *Salmonella thyphimurium* strains

| Tested compounds | Strains | | | | +0.02 M NMU |
|---|---|---|---|---|---|
| | TA98 | TA100 | TA1535 | TA1537 | |
| | Number of the $his^+$ revertants | | | | |
| 1 | 57 | 90 | 45 | 16 | 60 |
| 2 | 33 | 160 | 50 | 12 | 360 |
| 3 | 43 | 110 | 52 | 20 | 400 |
| 4 | 33 | 200 | 60 | 20 | 500 |
| 5 | 68 | 245 | 50 | 40 | 460 |
| 6 | 73 | 160 | 60 | 20 | 340 |
| 7 | 40 | 100 | 95 | 10 | 320 |
| Control | 53 | 100 | 40 | 22 | 280 |

Values presented are the means of 3 replicates, $P \leq 0.05$ [16].
All compounds were used in 1 mM concentration

## DISCUSSION

Our results demonstrate, that DL-p-chlorophenylalanine hydrochloride 1 in a DNA-repair test in bacteria, using 2 different techniques, did not exhibit genotoxicity and was devoid of mutagenic activity in the Ames reversion test. Meanwhile the methyl ester of this amino acid 2 was weakly genotoxic in spottest assay and was found to be non-mutagenic in the Ames test.

When DL-p-chlorophenylalanine was substituted at the N atom with sec-butyloxycarbonyl, benzyloxycarbonyl, p-nitrophenylacetyl or p-nitrophenoxyacetyl moieties, it was found that these compounds (3, 5, 6 and 7, respectively) possessed DNA damaging activity in the *Bacillus subtilis* rec assay. In the meanwhile, the introduction of the phenylacethyl moiety in the structure of this amino acid, the tested compound 4 exhibited only weak genotoxicity.

N-Acylderivatives 5 and 7 were mutagenic, since an increase in number of revertants was observed with respect to the untreated cells. TA100 and TA1535 in the presence of the compounds 5 and 7 were found to be the most sensitive strains. Furthermore, strain specificity indicates that the molecular mechanisms of above-mentioned compounds mutagenicity involve a base substitutions process. Only DL-p-chlorophenylalanine hydrochloride 1 possessed from seven tested compounds antimutagenic properties in respect of nitrosomethylurea. Antimutagenic effect of PCPA was dose-related: the increase of the NMU concentration caused the decrease of antimutagenic effect.

## CONCLUSION

Our findings demonstrate that PCPA is devoid genotoxic and mutagenic properties and has an antimutagenic effect that is favourable for use of this nonproteinogenic amino acid in clinical practice. Meanwhile the modification of the structure of PCPA led to compounds that significantly differed from genotoxic and mutagenic properties of parent amino acid. Such change of properties, may be due to the steric hindrance of the substituents, which interferes with enzyme and DNA interactions.

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