Synthesis, characterization, and activity of 6-(10H-phenothiazine-10-yl)-1H,3H-benzo[de]-isochromen-1,3-dione derivative of 4-aminophenylacetic acid

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Abstract. The objective of the present paper was to study the synthesis of a new compound 6-(10H-phenothiazine-10-yl)-1H,3H-benzo[de]-isochromen-1,3-dione derivative of 4-aminophenylacetic acid (PTZD). Its physicochemical characteristics and spectral data were presented. PTZD was found to have good inhibitory activity. The phenothiazine derivative showed good fungicidal activity against the studied yeast (S. cerevisiae and C. albicans) and mold species (A. brasiliensis and F. moniliforme), weak bactericidal activity against the Gram-positive bacteria S. aureus and B. subtilis and did not affect the growth of Gram-negative bacteria E. coli, P. aeruginosa and S. abony.

Keywords: phenothiazine, synthesis, corrosion, antimicrobial activity.

1. Introduction
Phenothiazines, nitrogen- and sulfur-containing tricyclic compounds have been known for more than a hundred years. The phenothiazine nucleus (10H-dibenzo-1,4-thiazine) was proven by Bernthsen in 1883. Since then, more than 5,000 phenothiazine derivatives have been synthesized. This class of organic compounds is extremely important due to its significant biological and chemical properties. Phenothiazines show good antimicrobial, antiprion, anthelmintic and insecticidal activity [1].

1,8-Naphthalene acid and many of its derivatives are intermediates in the synthesis of luminescent dyes and optical brighteners of polymeric materials [2, 3]. These include 4-halogen-1,8-naphthalene anhydrides. A peculiarity of their chemical structure is the presence in para position of a substituent in the 4-position of the peri-anhydride cycle, which has a solid electron acceptor effect, which gives halogen atoms great mobility in the nucleophilic substitution reaction. The reaction of 4-halogen-1,8-naphthalene anhydrides with secondary amines, such as phenothiazine, precludes the formation of imides, and the only reaction products are the corresponding anhydrides with nitrogen-containing substituents [4]. Ostis [5] increases the range of compounds, but the amines used are mostly alkylamines and single-nucleus heterocyclic amines. There are no amines with more nuclei, such as phenothiazine. Phenothiazine derivatives have a tranquilizing and fungicidal effect [6] and some combination action with the corresponding naphthalene cycle can be sought.
Some organic chemical compounds imported in insignificant quantities in a corrosive environment or in protective coatings (paints, varnishes, polymers, etc.) repeatedly reduce the rate of the corrosion process and reduce its harmful effects [7-9]. In recent years, significant progress has been made in the development of new and effective corrosion inhibitors [15, 16].

The protective action of inhibitors is associated with changes in the state of the metal surface and in the kinetics of the partial reactions underlying the corrosion process. A huge number of organic compounds have been studied as acid corrosion inhibitors [10, 11].

The aim of the present work is the synthesis of a phenothiazine derivative (PTZD) and the study of its anticorrosive (inhibitory) and antimicrobial activity.

2. Experimental

2.1. General

All used chemicals were purchased from Merck. The melting point was determined by a SMP-10 digital melting point apparatus. The purity of the compound was checked by thin layer chromatography on Kieselgel 60 F254, 0.2 mm Merck plates, eluent system (vol. ratio): benzene : ethanol = 5 : 1. The elemental analysis data were obtained with an automatic analyzer Carlo Erba 1106. The IR spectrum was taken on Perkin-Elmer FTIR-1600 spectrometer in KBr disc. The NMR spectra were obtained on Bruker Avance III HD (500.13 MHz for $^1$H and 125 MHz for $^{13}$C NMR) spectrometer. The chemical shifts are given in parts per million (δ) relative to tetramethylsilane as internal standard for spectra in DMSO-$d_6$ solutions.

2.2. Synthesis of 6-(10H-phenothiazine-10-yl)-1H,3H-benzo[de]-isochromen-1,3-dione derivative of 4-aminophenylacetic acid

The scheme for obtaining PTZD is presented in figure 1.

6-Phenothiazinyl-1,8-naphthalene anhydride (2.76 g/0.007 mol) and 4-aminophenylacetic acid (1.2 g/0.008 mol) in 20 mL of glacial acetic acid were refluxed for 4 hours. After cooling, the mixture was poured into 200 mL of cold water and allowed to stand for 24 hours. This was followed by filtration and recrystallization with absolute ethanol.

![Figure 1. Scheme for obtaining PTZD.](image-url)

2.3. Corrosion indicators of the phenothiazine derivative

2.3.1. Samples

The tested samples are made of EN - S235J2 steel plate. They are parallelepiped-shaped and have a work surface $\approx 30,01 \times 10^{-4} \text{ m}^2 \pm 0,0001 \times 10^{-4}$.

2.3.2. Corrosive medium

0.1M sulfuric acid solution was used as a corrosive medium in the experiments.

2.3.3. Inhibitor

The corrosion inhibitory action of the newly synthesized PTZD and the effect of its concentration on the corrosion process of steel were studied. The substance is insoluble in water and was introduced into
the corrosive medium in the form of ethanol solution. The studies were performed at room temperature (20 ± 1°C).

2.3.4. Method of study
A gravimetric method was used [12], which provides information on the corrosion rate (κ), but not on the metal dissolution mechanism.

The corrosion rate was calculated by the formula:

\[ k = \frac{m_0 - m}{S \times t} \left[ g \, m^{-2} h^{-1} \right], \]

where:
- \( m_0 \) [g] and \( m \) [g] are the weight of the metal before and after the experiment, respectively;
- \( S \) [m²] - the sample surface;
- \( t \) [h] - is the residence time (54 h) in a corrosive medium (0.1 M H₂SO₄).

The PTZD effectiveness as an inhibitor is determined by the degree of protection and the coefficient of inhibitory action (Y):

\[ Z = \left( \frac{k_0 - k}{k_0} \right) \times 100 \% \]

\[ Y = \frac{k_0}{k} \]

where: \( k_0 \) is the corrosion rate of the metal in the corrosive media without the addition of organic matter, and \( k \) is the corrosion rate of the metal in the corrosive media with the addition of organic matter.

All studies were performed under the same conditions three times and the results obtained are reproducible.

2.4. Antimicrobial activity of the phenothiazine derivative
The antimicrobial activity of PTZD was determined by the method of diffusion in agar and test microorganisms: Gram-positive bacteria *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NTCC 6017, yeast *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 2601, molds *Fusarium moniliforme* and *Aspergillus brasiliensis* ATCC 16404.

A 1% solution in solvent dimethyl sulfoxide (DMSO) was prepared from PTZD.

The experiments were performed on nutrient medium Tryptic soy agar (Merck) - for bacteria, and Sabouraud dextrose agar (Merck) for yeast and molds. Agar media are melted in a Koch apparatus. They are cooled to 50 - 48°C and are inoculated with 1% of the pre-prepared suspensions of the test microorganisms. 20 mL of inoculated media are poured into sterile petri dishes (Ø = 90 mm). The agar is allowed to solidify. A cork borer was used to punch holes (Ø = 8 mm) in the agar. 50µl of the pre-prepared solutions were added dropwise to each hole and, after 30 minutes of pre-infusion at room temperature, the petri dishes were placed in a thermostat at 37°C for 24 hours for the bacteria; at 28°C for 48 h for yeast and for 72 h for mold fungi [13].

After cultivation, the diameters of the zones of growth inhibition were measured in mm, as: up to 15 mm the microbial culture was weakly sensitive; from 15 to 25 mm - sensitive and over 25 mm - highly sensitive.

The experiments were performed in parallel with the control sample of the solvent, taking into account its action as well.

The antimicrobial activity was expressed as mean valued after three measurements.
3. Results and Discussion

3.1. Characterization of the phenothiazine derivative
Physicochemical characteristics and spectral data prove that the synthesized compound is PTZD. Yield: 3.3 g /90%; m.p.: 208-209°C; Rf = 0.68. Elemental analysis: Calculated: C – 72.71%, H – 3.81%, N – 5.30%, S – 6.07%; Found: C – 72.58%, H – 3.72%, N – 5.28%, S – 5.95%.

4. IR (KBr, cm⁻¹): 3256 (OH), 3061 (arom.) cm⁻¹, 2933 (aliph.) cm⁻¹, 1725, 1682 (C=O).

1H NMR (δ, DMSO-d₆, ppm): 3.49 (s, 2H, CH₂), 7.04-7.99 (m, 10H, CH), 12.35 (s, 1H, OH);

13C NMR (δ, DMSO-d₆, ppm): 184.1 (C=O), 175.2 (C=O), 116.6-147.0 (CH), 53.0 (CH₂);

13C DEPT 135 (δ, DMSO-d₆, ppm): 53.0 (CH₂), 138.8 (CH), 139.2 (CH), 142.5 (CH), 143.6 (CH), 145.3 (CH).

3.2. Corrosion inhibitory action of the phenothiazine derivative
The values obtained by the gravimetric method for the corrosion rate, the degree of protection of the tested steel and the coefficient of inhibitory action at different concentrations of PTZD are presented in Table 1.

Table 1. Corrosion performance of the phenothiazine derivative.

| PTZD inhibitor | PTZD concentration, [C, mol.L⁻¹] | Corrosion rate, \(\kappa, \text{gm}^{-2}.\text{h}^{-1}\) | Degree of protection, Z, % | Coefficient of inhibitory action, Y |
|----------------|----------------------------------|-----------------------------------------------|-----------------------------|-------------------------------------|
|                | 1x10⁻⁶                           | 1.8716                                        | 20.00                       | 1.25                                |
|                | 5x10⁻⁶                           | 1.6185                                        | 30.82                       | 1.45                                |
|                | 1x10⁻⁵                           | 0.9926                                        | 57.57                       | 2.36                                |
|                | 5x10⁻⁵                           | 0.4660                                        | 80.08                       | 5.02                                |
|                | 1x10⁻⁴                           | 0.3716                                        | 84.12                       | 6.30                                |

It is known from the literature [14-16] that the most effective inhibitors of acid corrosion are aromatic compounds containing N and O, as well as complex bonds. The newly synthesized PTZD has these elements in its structure. Table 1 shows that good values for the degree of protection in inhibited sulfuric acid solutions were obtained. As the concentration of PTZD increases, the corrosion rate decreases 5 times, the degree of protection increases 4 times and the coefficient of inhibitory action is 5 times higher. These results give us reason to believe that this phenothiazine derivative could be used as a corrosion inhibitor of steel.

3.3. Antimicrobial action of the phenothiazine derivative

Table 2. Antimicrobial action of the phenothiazine derivative.

| Test microorganisms                     | Inhibition zone diameter (mm) |
|----------------------------------------|------------------------------|
| Staphylococcus aureus ATCC 6538        | 12.2 ± 0.2                   |
| Bacillus subtilis ATCC 6633             | 11.3 ± 0.1                   |
| Escherichia coli ATCC 8739             | -                            |
| Pseudomonas aeruginosa ATCC 9027       | -                            |
| Salmonella abony NCTC 6017             | -                            |
| Saccharomyces cerevisiae ATCC 9763      | 18.5 ± 0.0                   |
| Candida albicans ATCC 10231            | 23.4 ± 0.2                   |
| Aspergillus brasiliensis ATCC16404      | 16.3 ± 0.3                   |
| Fusarium moniliforme                   | 20.6 ± 0.1                   |

"-" – not detected
The studied PTZD exhibits fungicidal action against the studied molds *A. brasiliensis* and *F. moniliforme* and yeast *S. cerevisiae* and *C. albicans*, the diameter of the growth inhibition zones was between 16.3 mm and 23.4 mm (Table 2). The antibacterial activity of the compound against Gram-positive bacteria *S. aureus* (12.2 mm) and *B. subtilis* (11.3 mm) is weak. The studied Gram-negative bacteria *E. coli*, *P. aeruginosa* and *S. abony* are not sensitive to its action.

4. Conclusions
1. A new compound – 6-(10H-phenothiazine-10-yl)-1H,3H-benzo[de]-isochromen-1,3-dione derivative of 4-aminophenylacetic acid was synthesized, not described in the literature so far.
2. PTZD has been found to have a good inhibitory effect. The corrosion rate of EN-S235J2 steel decreases with increasing the concentration of PTZD in sulfuric acid medium, the degree of protection and the coefficient of inhibitory action increase.
3. The phenothiazine derivative shows good fungicidal activity against the used yeasts (*S. cerevisiae* and *C. albicans*) and molds (*A. brasiliensis* and *F. moniliforme*), weak bactericidal activity against the Gram-positive bacteria *S. aureus* and *B. subtilis* and does not affect the Gram-negative bacteria *E. coli*, *P. aeruginosa* and *S. abony*.
4. The newly synthesized compound is suitable for protection against microbiological contamination and surface corrosion of machines and apparatus for the food industry.

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