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SYNTHESIS, CHARACTERIZATION, AND ACTIVITY OF PHENOTHIAZINE SCHIFF BASES CONTAINING FLUORINE

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Abstract. This paper presents the synthesis of a series of Schiff bases derived from phenothiazine derivatives with fluorine benzaldehydes. The compounds have been physicochemically characterized by NMR and IR spectroscopy, and their parameters have been determined by appropriate methods.
Their antimicrobial activity against various microorganisms has been studied. One of the compounds has shown a weak activity against *Bacillus subtilis* and *Salmonella abony*. The rest have not been active against microorganisms used.
The corrosion characteristics of the obtained compounds has been studied using electrochemical techniques (cyclic voltammetry, polarization curves and impedance spectroscopy) in a three-electrode corrosion cell relative to (hydrogen reference electrode) RHE. The samples have been applied to an experimental ground-graphite electrode using a spray technique with a thickness of about 50 μm. A detailed analysis of the internal resistance has been carried out, including equivalent model schemes and experimental data.

1. Introduction

Schiff bases compounds with azomethine functional group C=N are a significant category which has gained importance in various areas [1–3]. Phenothiazine and its derivatives possess potential biological activities as antinociceptive [4] anticonvulsant [5], antitumour [6], antimalarial [7], antitubercular [8], antiemetic [9], antihistaminic [10] and antipsychotic [11] agents. Increasing the resistance of microorganisms to currently available antimicrobial drugs is a major cause of morbidity and mortality throughout the world. Thus, the development of novel antimicrobial Schiff bases is still in demand [12]. In addition, phenothiazine moieties have been found frequently in other important molecules such as optoelectronic materials, antioxidants, polymerization inhibitors, industrial dyes and agrochemical compounds [13–15].

Schiff bases of phenothiazine derivatives with different chain inclusions have been reported in the literature [16-22], as well as coordination compounds [23]. They all have biological activity.

In the last year, several articles have been published in which a new derivative of phenothiazine with naphthalene nucleus, included in the chain, has been reported [24-26]. It is clear from these that the derivatives obtained demonstrate antimicrobial action [24, 25], as well as inhibitory action on acid corrosion [26].

A significant progress has been made over the recent years in developing new and effective corrosion inhibitors [27, 28].

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 large number of organic compounds have been studied as acid corrosion inhibitors [29, 30].

The aim of the current paper is to present the synthesis of Schiff bases of phenothiazine (PTZD) and the study of its anticorrosive (inhibitory) and antimicrobial activity.

2. Experimental

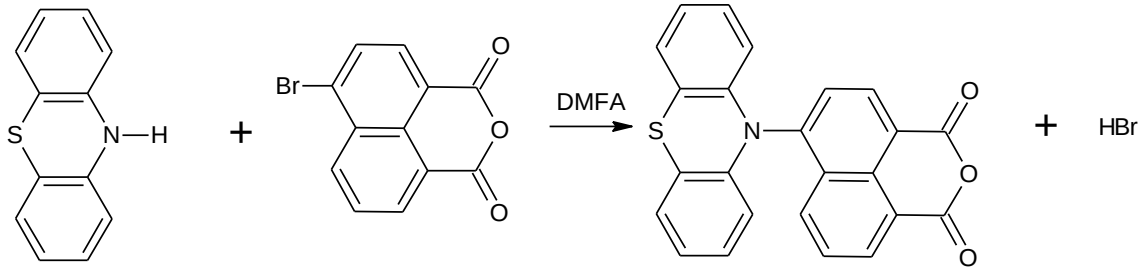
2.1. *General*

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 F_{254}, 0.2 mm Merck plates, eluent system (vol. ratio): benzene : ethanol = 5 : 1. The IR spectra were taken on Perkin-Elmer FTIR-1600 spectrometer in KBr discs. 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 Schiff bases

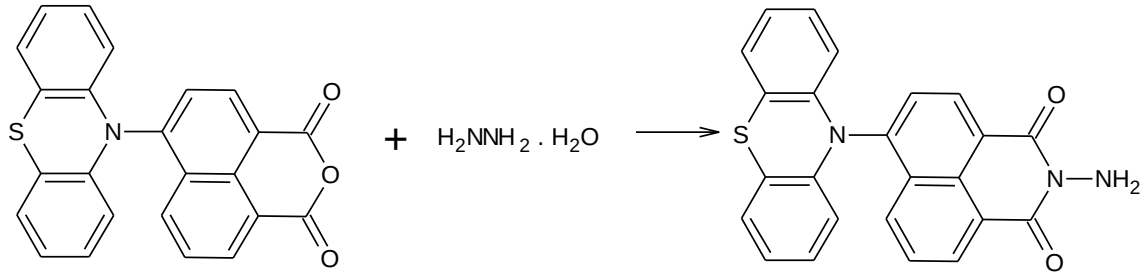
2.2.1. Synthesis of 6-(10H-phenothiazin-10-yl)-1H,3H-benzo[de]isochromene-1,3-dione

We used 1,8-naphthalene anhydride in the synthesis of Schiff bases, from which we obtain 4-bromonaphthalene anhydride [31]. The obtained anhydride was used to prepare 6-(10*H*-phenothiazin-10-yl)-1*H*, 3*H*-benzo[*de*]isochromene-1,3-dione according to the scheme:



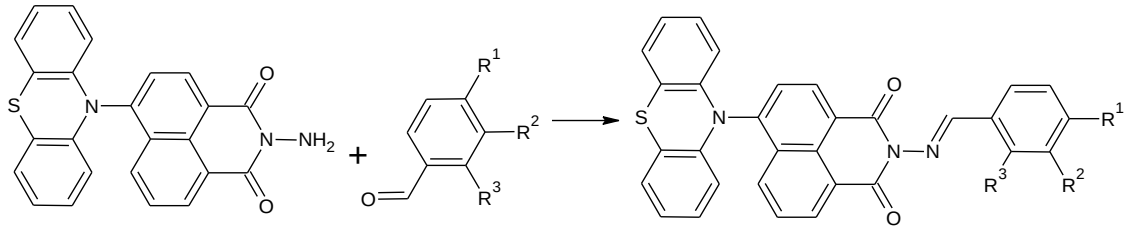
2.2.2. Synthesis of 2-amino-6-(10H-phenothiazin-10-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione

We obtained 2-amino-6-(10*H*-phenothiazin-10-yl))-1*H*-benzo[de]isoquinoline-1,3(2*H*)-dione from 6-(10*H*-phenothiazin-10-yl)-1*H*,3*H*-benzo[*de*]isochromene-1,3-dione with hydrazine hydrate ($H_2NNH_2.H_2O$) in accordance with the scheme below:



2.2.3. Synthesis of Schiff bases

0.005 mol of 2-amino-6-(10*H*-phenothiazin-10-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (2.05 g) was dissolved in 40 ml of CH_3OH and 0.005 mol of aldehyde was added. The mixture was heated on a water bath for 1h (~ 100 °C), cooled, crystallized and filtered under vacuum, then washed with CH_3OH. Thus, a pure product was obtained. The synthesis scheme is



where R^1, R^2, R^3 are F-atoms

	R^1	R^2	R^3
1-1	F	H	H
1-2	H	H	F
1-3	F	F	H

2.3. Corrosion indicators of the phenothiazine derivative

The corrosion behavior of the samples was investigated by impedance spectroscopy technic Gamry 1010E in OCP (open circuit mode) in the tree electrode liquid cell with 1M H_2SO_4 and cell temperature of 25°C. The counter electrode of the cell was a Pt wire with a thinness of 0.05mm and an area of about 10cm^2. As reference electrode were used commercial RHE (Reference Hydrogen Electrode) of the company Gaskatel. The samples dissolved in the isopropanol 96% in a form of ink and sprayed with loading of 1$mg.cm^{-2}$ over the highly conductive graphite support electrode. The active area of the investigated samples were 0.3cm^2.

2.4. Antimicrobial activity of the phenothiazine derivative

The antimicrobial activity of substances was determined by 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 was prepared in solvent dimethyl sulfoxide (DMSO) from the compounds.

The experiments were performed on nutrient medium Tryptic soy agar (Merck) - for bacteria, and Sabouraud dextrose agar (Merck) for yeast and molds. Agar media was melted in a Koch apparatus. They were cooled down to 50 - 48°C and inoculated with 1% of pre-prepared suspensions of the test microorganisms. 20 mL of inoculated media were poured into sterile petri dishes (∅ = 90 mm). The agar was left to solidify. A cork borer was used to punch holes (∅ = 8 mm) in the agar. 50µl of the preprepared 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 antimicrobial activity was valued after three measurements.

3. Results and discussion.

Three Schiff bases have been synthesized. The physicochemical data of the newly synthesized compounds are presented in the table 1.

Table 1. Characteristics of the obtained compounds

Compound	Melting point, °C	Yield, %	Molar mass	Molecular formula
1-1	223-224	45%	515,56	$C_{31}H_{18}FN_3O_2S$
1-2	256-257	37,1%	515,56	$C_{31}H_{18}FN_3O_2S$
1-3	202-203	43,7%	533,55	$C_{31}H_{17}F_2N_3O_2S$

The compounds obtained were characterized by NMR spectroscopy, the data of which are as follows:
Comp. 1-1
^{1}H NMR (δ, ppm, DMSO-d_6): 6,76-6,97 (m, 8H, phenothiazine), 7,47-8,78 (m, 5H, naphthalene), 7,00-7,43 (m, 4H, phenyl), 10,23 (s, 1H, benzylideneimine);
^{13}C NMR (δ, ppm, DMSO-d_6): 116,8-133,9 (CH, phenothiazine, naphthalene, phenyl), 161,6 (C=O), 168,6 (C=O), 154,2 (-CH=N);
^{13}C DEPT 135 (δ, ppm, DMSO-d_6): all C=O signals disappear.

Comp. 1-2
^{1}H NMR (δ, ppm, DMSO-d_6): 6,76-6,97 (m, 8H, phenothiazine), 7,47-8,78 (m, 5H, naphthalene), 7,00-7,43 (m, 4H, phenyl), 10,31 (s, 1H, benzylideneimine);
^{13}C NMR (δ, ppm, DMSO-d_6): 116,8-133,9 (CH, phenothiazine, naphthalene, phenyl), 162,5 (C=O), 169,1 (C=O), 155,3 (-CH=N);
^{13}C DEPT 135 (δ, ppm, DMSO-d_6): all C=O signals disappear.

Comp. 1-3
^{1}H NMR (δ, ppm, DMSO-d_6): 6,76-6,97 (m, 8H, phenothiazine), 7,47-8,78 (m, 5H, naphthalene), 7,00-7,43 (m, 4H, phenyl), 10,54 (s, 1H, benzylideneimine);
^{13}C NMR (δ, ppm, DMSO-d_6): 116,8-133,9 (CH, phenothiazine, naphthalene, phenyl), 163,1 (C=O), 169,5 (C=O), 156,6 (-CH=N);
^{13}C DEPT 135 (δ, ppm, DMSO-d_6): all C=O signals disappear.

The following results were obtained from the IR spectral studies performed, which prove the preparation of new compounds with expected structure

Table 2. IR spectroscopy data (KBr, cm^{-1})

Comp.	C=O	C=N	C-N	Arom.	C-F
1-1	1705 1652	1601	1342	3074	1236
1-2	1710 1671	1619	1337	3099	1233
1-3	1726 1704	1613	1341	3030	1236 1287

The corrosion behavior was investigated by impedance spectroscopy in a tree electrode corrosion cell in 1M H_2SO_4 at 25°C. The obtained results from the graphite support electrode are presented on the fig 1.

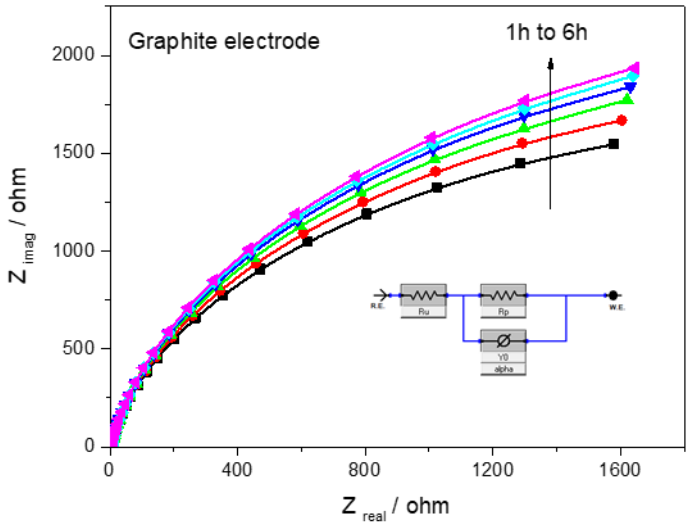


Fig. 1. Impedance measurements and equivalent scheme of the graphite support electrode at OCP in 1M H_2SO_4 and 25°C vs. RHE

The equivalent scheme shows classical additional resistance from the connected cables, and classical RC scheme. The real ohmic at the high frequency is comparatively low while the ohmic resistance of the low frequency increases up to 1600 ohms. The difference of the Zimag due to the time, is probably results from the increasing of the among of the 1M H_2SO_4 goes deep into the electrode surface. The results from all investigated samples are presented on fig. 2.

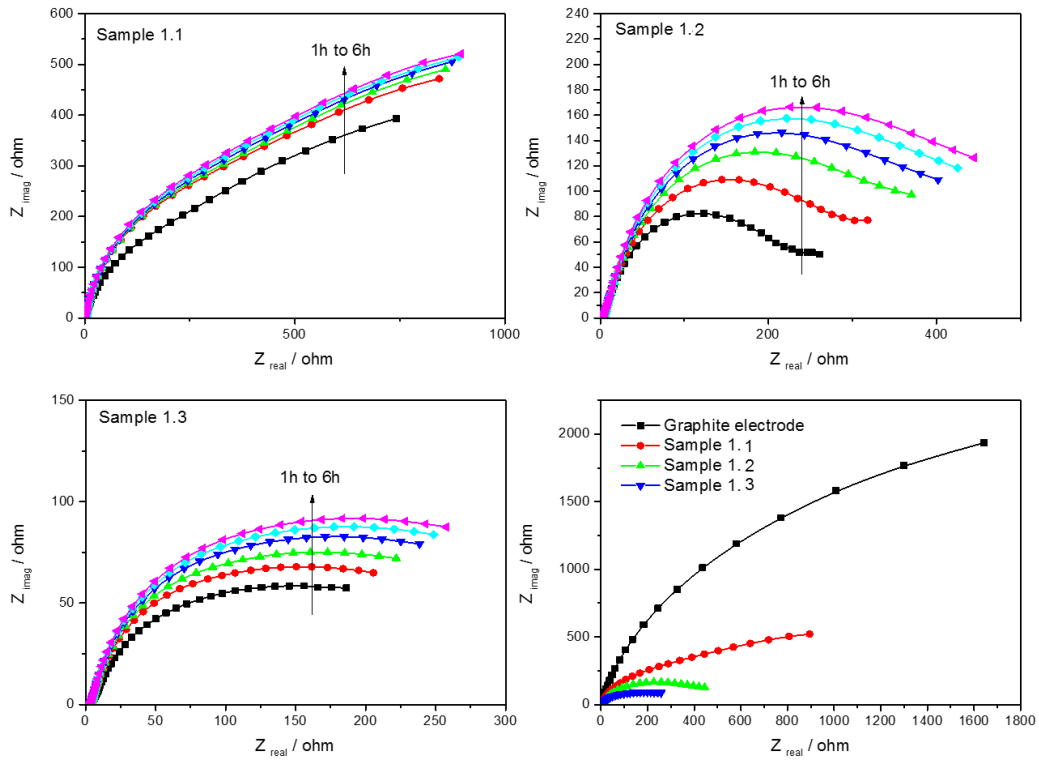


Fig. 2. Impedance measurements and equivalent scheme of the investigated samples strayed over the graphite support electrode at OCP in 1M H_2SO_4 and 25°C vs. RHE

The prepared samples were characterized in the same way as the graphite support material. The sample 1-1 showed difference between the 1st hour and the 6th hour. We suspect that during this time the corrosion of one of the elements inside was dissolved completely. After this time the sample demonstrated stable behavior and low changing of the real ohmic resistance. The second investigated sample 1-2 showed semi-circle and low degradation of the elements inside the layer. During this time the real ohmic resistance increased almost twice. The third sample 1-3 showed better ohmic resistance in comparison to the other two samples and, at the same time, lower level of degradation for the period of 6 hours. The comparison of all investigated sample and the graphite electrode are presented on the last graph of fig. 2. It confirms that sample 1-3 demonstrated a high stability in comparison with all investigated samples and a better charge transfer.

The data from the antimicrobial test are given in Table 3.

Table 3. Data from the antimicrobial study of the obtained compounds

Microorganisms	Zone diameter (mm)		
	1-1	1-2	1-3
Staphylococcus aureus ATCC 6538	0	0	0
Bacillus subtilis ATCC 6633	0	0	10,5
Escherichia coli ATCC 8739	0	0	0
Pseudomonas aeruginosa ATCC 9027	0	0	0
Salmonella abony NCTC 6017	0	0	11,0
Saccharomyces cerevisiae ATCC 2601	0	0	0
Candida albicans ATCC 10231	0	0	0
Aspergillus brasiliensis ATCC 16404	0	0	0
Fusarium moniliforme	0	0	0

The data show that compound 1-3 is weakly active against the Gram-positive bacterium *B. subtilis* and the Gram-negative bacterium *S. abony* and inactive against the bacteria *S. aureus*, *E. coli*, *P. aeruginosa* and the yeasts and molds used. Compounds 1-1 and 1-2 are inactive against all test microorganisms used.

4. Conclusions

1. Three new compounds have been synthesized, as detected by IR and NMR spectroscopy
2. Physicochemical parameters of the compounds obtained have been determined
3. Al investigated samples demonstrate stability in the 1M H_2SO_4 for the period of 6 hours.
4. Sample 1-3 shows lower ohmic resistance and improved charge transfer in comparison with 1-1 and 1-2. The ohmic resistance of sample 1-3 is comparatively better than the other investigated samples.
5. Compound 1-3 is weakly active against the Gram-positive bacterium *B. subtilis* and the Gram-negative bacterium *S. abony* and inactive against the bacteria *S. aureus*, *E. coli*, *P. aeruginosa* and the yeasts and molds used.
6. Compounds 1-1 and 1-2 are inactive against all test microorganisms used.

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