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Phenothiazine Schiff bases containing chlorine: synthesis, characterization and antimicrobial study

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This article presents the synthesis, structural and spectral (IR and NMR) characterization of some new phenothiazine Schiff bases containing chlorine. The target compounds (Figs. 1a-1d) were obtained as a result of the reaction between 2-amino-6-(10*H*-phenothiazin-10-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione and the corresponding chloro-benzaldehydes. The antimicrobial activity of the synthesized Schiff bases was evaluated against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella abony*, yeasts *Saccharomyces cerevisiae* and *Candida albicans*, molds *Aspergillus brasiliensis* and *Fusarium moniliforme*.

Keywords: phenothiazine, Schiff bases, antimicrobial activity

INTRODUCTION

Compounds containing the -C=N- (azomethine) group in their structure are known as Schiff bases, and are synthesized by condensation of primary amines and active carbonyl compounds [1]. Schiff bases are known for a broad range of biological activities, such as antitubercular [2], anticancer [3-5], analgesic [6, 7], anti-inflammatory [8, 9], anticonvulsant [10, 11], antibacterial [12], antifungal [13] antioxidant [14, 15], antitumor [16, 17] and anthelmintic [18] activities.

In previous studies of ours, we have presented the synthesis [19, 20], and studies of antimicrobial and corrosion inhibition properties of some phenothiazine products. The results of these studies have prompted us to continue working in this direction, and to synthesize and study new compounds from the group of phenothiazine Schiff bases, not previously described in the literature.

MATERIALS AND METHODS

All used chemicals were purchased from Merck and Sigma-Aldrich. The melting points were determined on a SMP-10 digital melting point apparatus. The IR spectra were taken on Perkin-Elmer FTIR-1600 spectrometer in KBr discs. The NMR spectra were taken on a Bruker DRX-250 spectrometer operating at 250.13 and 62.90 MHz for ^{1}H and ^{13}C, respectively, using the standard Bruker software. The chemical shifts were referenced to tetramethylsilane (TMS). The measurements in DMSO-d_6 solutions were carried out at ambient temperature (300 K).

EXPERIMENTAL

The synthesis of the initial compound 2-amino-6-(10*H*-phenothiazin-10-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione is presented in Scheme 1 [21].

Synthesis of Schiff bases containing chlorine (Scheme 2)

0.005 mol of 2-amino-6-(10*H*-phenothiazin-10-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (1) is dissolved in 40 mL of MeOH and 0.005 mol of the corresponding chlorobenzaldehyde (2a-2d) is added. The reaction mixture is heated in a water bath for 1 h (~100°C). After cooling, the product formed (3a-3d) is filtered off and washed with MeOH.

Antimicrobial study

Agar diffusion method 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 *Aspergillus brasiliensis* ATCC 16404 and *Fusarium moniliforme*, were used to determine the antimicrobial action of the synthesized compounds. 1% solutions in solvent dimethyl sulfoxide (DMSO) were prepared from the compounds tested.

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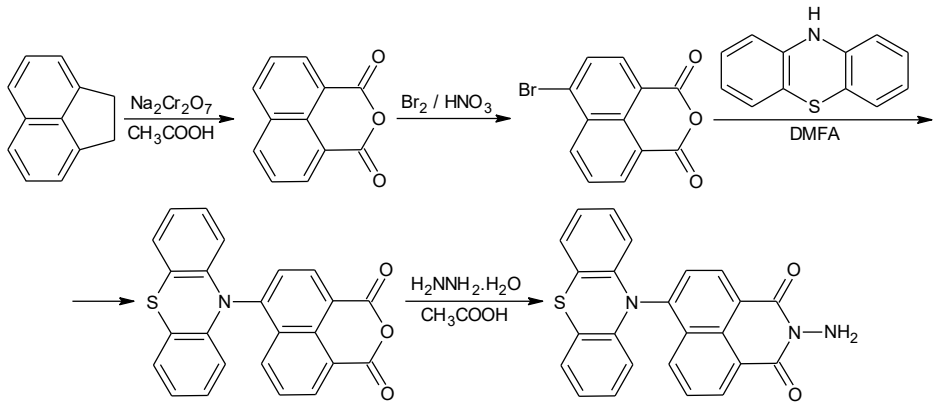
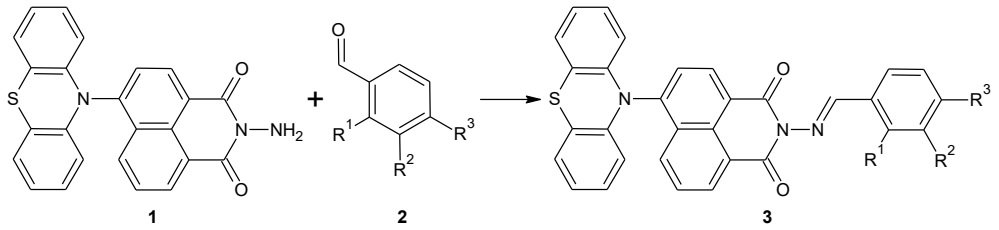


Scheme 1



a) R^1 = Cl, R^2 = R^3 = H; b) R^1 = R^2 = Cl, R^3 = H; c) R^1 = R^3 =Cl, R^2 = H;
d) R^1 = R^2 = H, R^3 = Cl

Scheme 2

The experiments were performed in nutrient medium Tryptic soy agar (Merck) for bacteria and Sabouraud dextrose agar (Merck) for yeast and molds.

The agar media were melted in a Koch apparatus. They were cooled down to a temperature of 50-48°C and inoculated with 1% of the prepared suspensions of the test microorganisms, then mixed well. 20 mL of the inoculated media were poured into sterile Petri dishes (Ø=90 mm). The agar was allowed to solidify. Cork borer was used to punch holes (Ø=8 mm) in the agar plate. 50 µL of the prepared solutions were added dropwise to the wells, and after 30 min of prefusion at room temperature, the petri dishes were placed in a thermostat at 37°C for 24 h for bacteria; 28°C for 24 h for yeasts and for 72 h for molds [22]. Diameters of the zones of growth inhibition were taken into account after cultivation as follows: up to 15 mm the microbial culture is weakly sensitive; from 15 to 25 mm – sensitive and over 25 mm – highly sensitive.

RESULTS AND DISCUSSION

Four Schiff base derivatives of phenothiazine with the structure shown in Fig. 1 were synthesized in accordance with Scheme 2 (see the Experimental part).

The yields and melting points of the obtained compounds (3a-3d) are given in Table 1. The spectral data (IR, ^{1}H NMR and ^{13}C NMR including ^{13}C DEPT 135) of the compounds 3a-3d are presented in Tables 2-4.

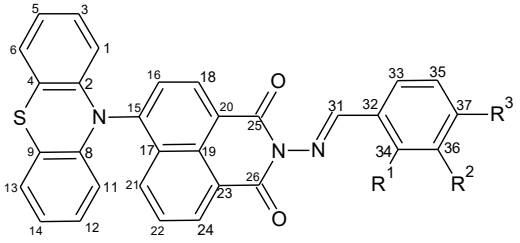


Figure 1. a) R^1 = Cl, R^2 = R^3 = H; b) R^1 = R^2 = Cl, R^3 = H; c) R^1 = R^3 =Cl, R^2 = H; d) R^1 = R^2 = H, R^3 = Cl (The numbering of the atoms is only for spectral assignments)

Table 1. Yields and melting points

№	Systematic name	M. p., °C	Yield, %
3a	2-[(2-hlorophenyl)methylene-amino]-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione	204-205	30
3b	2-[(2,3-dichlorophenyl) methyleneamino]-6-phenothiazin-10-yl-benzo [*de*]isoquinoline-1,3-dione	163-164	46
3c	2-[(2,4-dichlorophenyl) methyleneamino]-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione	206-207	46
3d	2-[(4-chlorophenyl)methylene-amino]-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione	199-200	38

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

№	*v* C=O	*v* C=N	*v* C-N	*v* Arom.	*v* C-Cl
3a	1707, 1697	1612	1340	3089	775
3b	1704, 1671	1609	1338	3061	774, 741
3c	1704, 1671	1613	1340	3090	774, 741
3d	1703, 1654	1618	1341	3034	778

Table 3. Selected ^{1}H NMR spectroscopic data (DMSO-d_6, δ, ppm)

№	
3a	5.80-7.69 (m, 8H, CH, phenoth. core), 8.01-8.24 (m, 4H, CH, benz. core), 8.28 (s, 1H, CH), 8.34-8.77 (m, 5H, CH, naphth. core)
3b	6.67-6.75 (m, 8H, CH, phenoth. core), 6.90-6.99 (t, 3H, CH, benz. core), 8.27 (s, 1H, CH), 8.29-8.64 (m, 5H, CH, naphth. core)
3c	5.80-6.91 (m, 8H, CH, phenoth. core), 6.98-8.05 (t, 3H, CH, benz. core), 8.28 (s, 1H, CH), 8.38-8.64 (m, 5H, CH, naphth. core)
3d	5.80-6.91 (m, 8H, CH, phenoth. core), 6.98-8.00 (t, 3H, CH, benz. core), 8.25 (s, 1H, CH), 8.35-8.80 (m, 5H, CH, naphth. core)

Table 4. Selected ^{13}C NMR and ^{13}C DEPT 135 spectroscopic data (DMSO-d_6, δ, ppm)

Position	Compounds							
	3a	3b	3c	3d	3a	3b	3c	3d
	^{13}C NMR				^{13}C DEPT-135			
1|11	122.2	122.2	114.9	129.5	CH	CH	CH	CH
2|8	135.5	135.5	142.6	143.3				
3|12	132.4	133.9	132.1	129.8	CH	CH	CH	CH
4|10	123.7	122.9	132.2	131.7				
5|14	128.5	126.7	129.6	131.8	CH	CH	CH	CH
6|13	128.7	129.6	128.0	132.0	CH	CH	CH	CH
15	134.8	128.0	133.9	137.9				
16	122.9	116.2	122.2	114.9	CH	CH	CH	CH
17	123.7	125.5	126.7	129.9				
18	133.3	130.5	132.9		CH	CH	CH	CH
19	129.4	130.6	130.6					
20	129.9	132.2	122.9					
21	130.2	132.9	133.9	130.9	CH	CH	CH	CH
22	130.4	132.2	133.9	132.5	CH	CH	CH	CH
23	131.5	133.2	122.2					
24	131.8			133.5	CH		CH	CH
25|26	160.3	161.7	161.7	160.3				
31	168.6	168.6	168.6	171.3	CH			
32	131.9	131.2	116.8	128.5				
33	130.8	118.7		121.4	CH	CH	CH	CH
34	131.9	133.6	134.2	121.4				CH
35	132.1	129.4		125.5	CH	CH	CH	CH
36	122.1	133.8	136.6	125.5	CH		CH	CH
37	133.4				CH	CH		

Table 5. Antimicrobial activity of compounds 3a-3d

Test microorganisms	Diameter of the inhibition zone (mm)			
	3a	3b	3c	3d
Staphylococcus aureus ATCC 6538	0	0	0	0
Bacillus subtilis ATCC 6633	10.4	0	0	12.8
Escherichia coli ATCC 8739	0	0	0	0
Pseudomonas aeruginosa ATCC 9027	0	0	0	0
Salmonella abony NTCC 6017	0	0	0	11.6
Saccharomyces cerevisiae ATCC 2601	0	0	0	0
Candida albicans ATCC 10231	0	0	0	0
Aspergillus brasiliensis ATCC 16404	0	0	0	0
Fusarium moniliforme	0	0	0	0

The IR and ^{1}H-NMR spectral data of the compound 2-amino-6-(10*H*-phenothiazin-10-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione [23] show the signals for NH_2 group as follows: 3441 cm^{-1} and 3324 cm^{-1} and 2.51 ppm and 3.35 ppm. These signals are absent for the compounds 3a-d. In the synthesized Schiff bases, signals for >C=N group appear in the region 1609-1618 cm^{-1}. The ^{1}H-NMR spectra of the compounds showed signals for a CH group (№ 31, Figure 1) in the region 8.25-8.28 ppm. This confirms the interaction between the corresponding amine and the aromatic aldehyde leading to the formation of the Schiff bases 3a-d. In the ^{13}C-NMR spectra, a new signal for the >C=N group appears, which for compounds 3a-c is around 168.6 ppm, only in compound 3d it is at 171.3 ppm, which is most likely due to the *p*-chlorine atom in the aromatic aldehyde.

Compound 3a shows weak antibacterial activity against the Gram-positive bacterium *Bacillus subtilis*. Compound 3d demonstrates weak activity against *Bacillus subtilis* and the Gram-negative bacterium *Salmonella abony* (Table 5). No activity of these compounds was established against any other test microorganisms used. Compounds 3b and 3c show no activity towards the microorganisms used.

CONCLUSION

Four new phenothiazine derivatives, previously not described in the literature, have been synthesized and their structures have been proven by IR and ^{1}H, ^{13}C and DEPT-135 spectroscopy. Their antimicrobial activity has been investigated, where two of the compounds have shown weak activity against *Bacillus subtilis*, and one is also active against *Salmonella abony*. Two of the compounds have demonstrated no activity towards the microorganisms used.

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