Synthesis and antimicrobial study of 6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione derivatives

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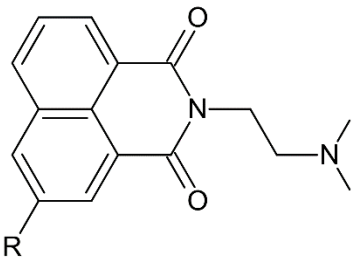
Abstract. This study presents the synthesis of various new 6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione derivatives. The structures of the synthesized compounds were confirmed through physicochemical analyses, IR, ^{1}H NMR, ^{13}C NMR, and ^{13}C DEPT 135 spectral data. The antimicrobial activity of the compounds was evaluated against Gram-positive and Gram-negative bacteria, as well as yeasts and molds. The products were found to exhibit weak to good activity against the tested Gram-positive and some Gram-negative bacterial strains.

1 Introduction

Several 1,8-naphthalimide derivatives with the general structural formula shown in Fig. 1 have been reported in the literature.



R = H; NO_2; NH_2 [1, 2]

Fig. 1. 1,8-Naphthalimide derivatives

The nitro and amino derivatives, known in clinical practice as mitonafide and amonafide, exhibit high antitumor activity with IC_{50} values of 0.47 nM and 8.8 nM, respectively, against Heba cell lines [3]. In addition to their antitumor effects, naphthalimide derivatives display a

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wide range of biological properties, including antitrypanosomal, antiviral, antimicrobial, and antioxidant activities [4, 5]. The synthesis of phenothiazine-substituted derivatives can be considered a rational approach to combining the key structural features of established naphthalimide-based anticancer agents with the biologically active phenothiazine moiety.

Naphthalimide is a polycyclic amide consisting of a planar, π-deficient aromatic or heteroaromatic system characterized by high hydrophobicity. Structure–activity relationship (SAR) studies indicate that specific structural parameters are critical for the antitumor activity of naphthalimide derivatives. Notably, the presence of a two- or three-methylene spacer between the naphthalimide core and the terminal amino group in the side chain appears to be essential for potent anticancer activity [3].

The present study is focused on the synthesis of hybrid structures based on these principles, as well as on the investigation of their antimicrobial potential.

2 Materials and methods

2.1 General

All chemicals used were obtained from Merck and Sigma-Aldrich. Melting points were measured using an SMP-10 digital melting point apparatus. The IR spectra were recorded on a Perkin-Elmer FTIR-1600 spectrometer using KBr disks. The NMR spectra were obtained with a Bruker Avance III HD spectrometer (operating at 500.13 MHz for ^{1}H and 125 MHz for ^{13}C) in DMSO-d_6 solutions. The chemical shifts were referenced to tetramethylsilane (TMS).

2.2 Synthesis of 2-(2-aminoethyl)-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione (IV)

To a continuously stirred solution of 1,2-diaminoethane (2.2 mL, 0.033 mol) in 12.5 mL of water, a suspension of 6-(10*H*-phenothiazin-10-yl)-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (III, 1.97 g, 0.005 mol) in 5 mL of water was added portionwise over 10 minutes at 75 °C. The resulting suspension was maintained at this temperature for 40 minutes, then hot-filtered, washed with water, and dried under vacuum. The crude solid was extracted with boiling chloroform, and after vacuum distillation of the solvent, the target product was obtained.

2.3 Synthesis of 2-(2-hydroxyethyl)-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione (V)

To a vigorously stirred suspension of compound III (3.95 g, 0.01 mol) in 15 mL of dimethylformamide, 2-aminoethanol (0.61 g, 0.01 mol) was added dropwise. The reaction mixture was refluxed for 1 hour, cooled to room temperature, and poured into 300 mL of cold water. After standing overnight, the precipitate was filtered, dried, and recrystallized from ethanol.

2.4 Synthesis of 2-[2-[(*E*)-benzylideneamino]ethyl]-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione (VIa)

A mixture of compound IV (4.37 g, 0.01 mol), benzaldehyde (1.02 mL, 0.01 mol), and glacial acetic acid (2 mL) in methanol (50 mL) was refluxed for 8 hours. After cooling, the reaction mixture was poured into 300 mL of cold water. The precipitated product was filtered and recrystallized from ethanol.

2.5 Synthesis of 2-[2-[(*E*)-(4-fluorophenyl)methyleneamino]ethyl]-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione (VIb)

The procedure was identical to that described in Section 2.4, using 4-fluorobenzaldehyde (0.01 mol) instead of benzaldehyde.

2.6 Synthesis of 2-[2-[(*E*)-(3,4-difluorophenyl)methyleneamino]ethyl]-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione (VIc)

The method followed the procedure in Section 2.4, using 3,4-difluorobenzaldehyde (1.1 mL, 0.01 mol) in place of benzaldehyde.

2.7 Synthesis of 2-(1,3-dioxo-6-phenothiazin-10-yl-benzo[*de*]isoquinolin-2-yl)ethyl 4-methylbenzenesulfonate (VII)

A suspension of compound V (1.88 g, 4.3 mmol) in 50 mL of dry pyridine was stirred at 0 °C (ice bath) for 15 minutes. To this, p-toluenesulfonyl chloride (1.2 g, 6.4 mmol) was added slowly over 30 minutes. The reaction mixture was kept at 4 °C for 12 hours. It was then poured into 200 mL of cold water and stirred for an additional 2 hours. The resulting solid was filtered, washed with water, and dried at 50 °C. The crude product was recrystallized from ethanol.

2.8 Determination of antimicrobial activity

The antimicrobial activity of the synthesized compounds was evaluated using the agar diffusion method. The test panel included Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, and *Bacillus cereus* ATCC 10876; Gram-negative bacteria: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, and *Salmonella abony* NTCC 6017; yeasts: *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763; and molds: *Aspergillus brasiliensis* ATCC 16404 and *Fusarium moniliforme* [6].

3 Results and discussion

The compound 6-(10*H*-phenothiazin-10-yl)-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (III) [7] was synthesized by the reaction of 6-bromo-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (I) with 10*H*-phenothiazine (II), as illustrated in Fig. 2.

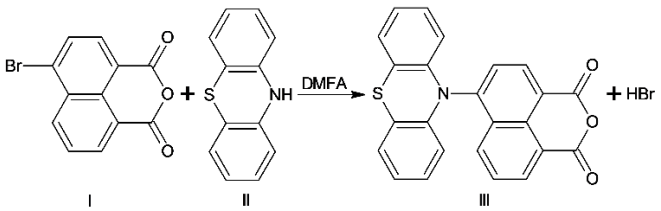


Fig. 2. Synthesis of compound III

Upon treatment of compound (III) with ethane-1,2-diamine and 2-aminoethan-1-ol, the corresponding derivatives 2-(2-aminoethyl)-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione (IV) and 2-(2-hydroxyethyl)-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione (V) were obtained, respectively (Fig. 3).

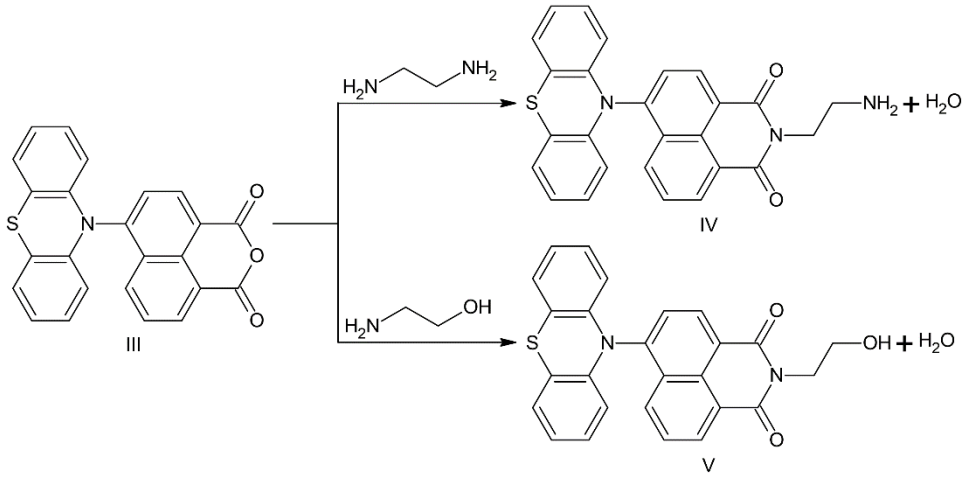
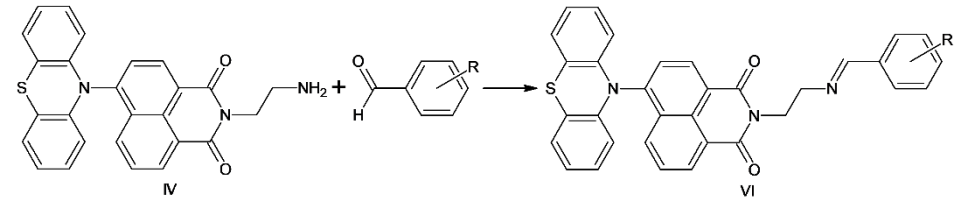


Fig. 3. Synthesis of compounds IV and V

Compound (IV) underwent condensation with aromatic aldehydes (Fig. 4) to afford the corresponding azomethine (Schiff base) derivatives (VI).



R = H (VIa); 4-F (VIb); 3,4-diF (VIc)

Fig. 4. Synthesis of compounds VIa-c

Additionally, compound (V) was reacted with p-toluenesulfonyl chloride in pyridine (Fig. 5), resulting in the formation of the corresponding benzenesulfonate derivative (VII).

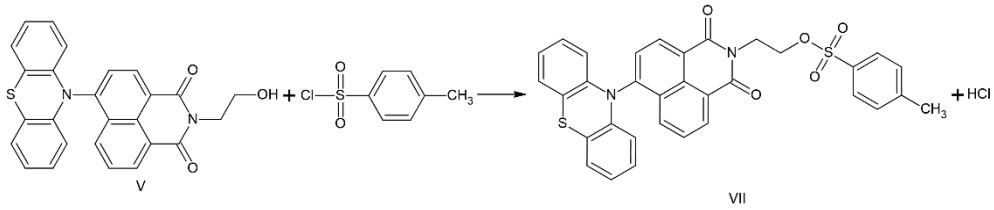


Fig. 5. Synthesis of compound VII

Six novel compounds were synthesized with yields ranging from 52% to 87% (Table 1). Their IR and NMR spectral data are summarized in Tables 2 and 3, respectively. The results of the antimicrobial activity studies are presented in Table 4.1

The data in Table 4 indicate that compounds IV and V exhibited good activity against the Gram-negative bacterium *Escherichia coli* (21.7 mm and 20.5 mm, respectively), and the Gram-positive bacterium *Bacillus subtilis* (18.3 mm and 21.2 mm, respectively). Moderate activity was observed against *Bacillus cereus* (15.5 mm and 14.2 mm) and *Staphylococcus epidermidis* (14.6 mm and 13.2 mm). Activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was lower (13.0 mm and 10.5 mm; 11.1 mm and 12.3 mm, respectively).

Table 1. Physicochemical parameters of compounds IV-VII

№	Systematic name	Yield, %	M. p., °C
IV	2-(2-aminoethyl)-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione	78	138-139
V	2-(2-hydroxyethyl)-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione	53	203-204
VIa	2-[2-[(*E*)-benzylideneamino]ethyl]-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione	71	281-282
VIb	2-[2-[(*E*)-(4-fluorophenyl)methyleneamino]ethyl]-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione	52	243-244
VIc	2-[2-[(*E*)-(3,4-difluorophenyl)methyleneamino]ethyl]-6-phenothiazin-10-yl-benzo[*de*]isoquinoline-1,3-dione	61	262-263
VII	2-(1,3-dioxo-6-phenothiazin-10-yl-benzo[*de*]isoquinolin-2-yl)ethyl 4-methylbenzenesulfonate	87	128-129

Compound VIc demonstrated significant activity against *Bacillus subtilis* (23.1 mm) and moderate inhibition of *Escherichia coli* (15.1 mm). It exhibited weak activity against *Bacillus cereus* (12.4 mm) and *Staphylococcus epidermidis* (11.6 mm), and was inactive against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Compound VIa showed antibacterial effects against *Bacillus subtilis* (17.6 mm) and *Escherichia coli* (19.6 mm). Lower activity was observed against *Bacillus cereus* (13.7 mm), *Staphylococcus epidermidis* (15.1 mm), *Staphylococcus aureus* (9.2 mm), and *Pseudomonas aeruginosa* (13.1 mm).

Compound VIb was active against *Escherichia coli* (18.9 mm), with modest activity against *Bacillus cereus* (12.5 mm) and *Staphylococcus epidermidis* (9.8 mm). No activity was detected against *Staphylococcus aureus* or *Pseudomonas aeruginosa*.

The IR, ^{1}H NMR, ^{13}C NMR, and ^{13}C DEPT 135 spectral data, summarized in Tables 2 and 3, unequivocally confirm the proposed structures of compounds IV-VII.

Table 2. IR spectral data (KBr, cm^{-1}) of compounds IV-VII

№	$\vartheta_{aliph.}$	$\vartheta_{arom.}$	ϑ_{NH_2}	$\vartheta_{C=O}$	ϑ_{OH}	$\vartheta_{C=N}$	ϑ_{C-N}
IV	2888	3068	3261, 3148	1696, 1666	-	-	-
V	2885	3065	-	1694, 1663	3392	-	-
VIa	2847	3067	-	1699, 1657	-	1589	1347
VIb	2850	3068	-	1700, 1658	-	1591	1346
VIc	2861	3065	-	1705, 1671	-	1605	1351
VII	2885	3059	-	1693, 1668	-	-	-

Compound VII showed antibacterial effects against *Bacillus cereus* (17.3 mm) and *Escherichia coli* (19.8 mm), and moderate activity against *Staphylococcus epidermidis* (15.2 mm), *Bacillus subtilis* (14.1 mm), and *Pseudomonas aeruginosa* (15.6 mm). Activity against *Staphylococcus aureus* was low (11.8 mm).

Table 3. NMR (DMSO-d_6, δ, ppm) spectral data of compounds IV-VII

№	NMR (DMSO-d_6, δ, ppm) spectral data of compounds IV-VII
IV	^{1}H NMR (DMSO-d_6, δ, ppm): 8.02-8.81 (m, 5H, CH arom./BZ core), 6.74-6.98 (m, 8H, CH arom./PTZ core), 4.14 (d, 2H, CH_2), 2.92 (d, 2H, CH_2), 1.23 (d, 2H, NH_2). ^{13}C NMR (DMSO-d_6, δ, ppm): 174.7, 153.7, 143.2, 142.4, 141.5, 141.2, 139.5, 139.3, 139.2, 137.7, 134.6, 133.7, 133.3, 117.9, 116.0, 54.2, 52.4. ^{13}C DEPT 135 (DMSO-d_6, δ, ppm): 143.2, 141.9, 141.5, 142.4, 139.5, 139.3, 137.6, 133.7, 116.0, 54.2, 52.4.
V	^{1}H NMR (DMSO-d_6, δ, ppm): 8.16-8.50 (m, 5H, CH arom./BZ core), 7.92-8.15 (m, 8H, CH arom./PTZ core), 4.83 (d, 2H, CH_2), 4.12 (d, 2H, CH_2), 1.17 (s, H, OH). ^{13}C NMR (DMSO-d_6, δ, ppm): 162.3, 141.3, 132.0, 131.9, 131.7, 131.3, 130.9, 130.7, 129.2, 129.1, 129.0, 128.5, 128.4, 128.1, 127.6, 122.2, 121.4, 58.2, 42.4. ^{13}C DEPT 135 (DMSO-d_6, δ, ppm): 131.9, 131.7, 131.3, 129.2, 129.1, 129.0, 128.5, 122.2, 121.4, 58.2, 42.4.
VIa	^{1}H NMR (DMSO-d_6, δ, ppm): 10.02 (s, H, CH), 7.61-8.71 (m, 5H, CH arom./NAP core), 7.20-7.60 (m, 5H, CH arom./BZ core), 6.67-7.00 (m, 8H, CH arom./PTZ core), 4.46 (d, 2H, CH_2), 3.93 (d, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ, ppm): 163.4, 162.8, 141.9, 136.4, 133.1, 132.1, 131.9, 131.5, 131.2, 130.3, 130.0, 129.3, 129.1, 128.4, 127.1, 126.7, 123.2, 120.2, 58.1, 40.8. ^{13}C DEPT 135 (DMSO-d_6, δ, ppm): 162.8, 133.1, 132.1, 131.9, 131.5, 131.2, 129.3, 129.1, 128.4, 126.7, 120.2, 58.1, 40.8.
VIb	^{1}H NMR (DMSO-d_6, δ, ppm): 9.98 (s, H, CH), 7.83-8.57 (m, 5H, CH arom./NAP core), 7.73-7.76 (m, 4H, CH arom./BZ core), 6.57-7.23 (m, 8H, CH arom./PTZ core), 4.31 (d, 2H, CH_2), 3.90 (d, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ, ppm): 163.3, 163.2, 161.5, 141.2, 133.1, 133.0, 132.1, 131.8, 131.6, 131.4, 130.6, 130.5, 130.2, 129.7, 129.6, 129.3, 128.7, 123.1, 122.5, 122.4, 116.2, 116.0, 58.0, 40.8. ^{13}C DEPT 135 (DMSO-d_6, δ, ppm): 161.5, 133.1, 132.1, 131.8, 131.6, 131.4, 130.6, 130.5, 129.6, 129.3, 116.2, 116.0, 58.0, 40.8.
VIc	^{1}H NMR (DMSO-d_6, δ, ppm): 9.12 (s, H, CH), 8.22-8.59 (m, 5H, CH arom./NAP core), 7.48-7.55 (t, 3H, CH arom./BZ core), 6.67-6.97 (m, 8H, CH arom./PTZ core), 4.33 (d, 2H, CH_2), 3.86 (d, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ, ppm): 163.5, 160.9, 142.5, 139.6, 139.4, 135.4, 134.7, 133.8, 133.3, 133.1, 132.3, 131.8, 131.5, 129.3, 128.1, 128.0, 126.8, 123.3, 122.2, 118.4, 116.7, 115.0, 112.4, 57.9, 40.7. ^{13}C DEPT 135 (DMSO-d_6, δ, ppm): 160.9, 133.3, 132.3, 131.8, 129.3, 128.1, 126.8, 122.2, 118.4, 116.7, 115.0, 112.4, 57.9, 40.7.
VII	^{1}H NMR (DMSO-d_6, δ, ppm): 7.98-8.65 (m, 4H, CH arom./BZ core), 6.63-6.88 (m, 8H, CH arom./PTZ core), 4.46 (d, 2H, CH_2), 4.12 (d, 2H, CH_2), 2.24 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 164.3, 154.1, 142.2, 141.4, 140.3, 139.6, 139.4, 139.2, 138.8, 138.5, 135.6, 134.3, 134.2, 118.2, 117.3, 68.5, 38.8, 22.1. ^{13}C DEPT 135 (DMSO-d_6, δ, ppm): 68.5, 38.8, 22.1.

Table 4. Antimicrobial activity of compounds IV-VII

Test microorganism	Inhibition zone diameter (mm)					
	IV	**V**	**VIa**	**VIb**	**VIc**	**VII**
Staphylococcus aureus ATCC 6538	13	10.5	9.2	0	0	11.8
Staphylococcus epidermidis ATCC 12228	14.6	13.2	15.1	9.8	11.6	15.2
Bacillus subtilis ATCC 6633	18.3	21.2	17.6	14.8	23.1	14.1
Bacillus cereus ATCC 10876	15.5	14.2	13.7	12.5	12.4	17.3
Escherichia coli ATCC 8739	21.7	20.5	19.6	18.9	15.1	19.8
Pseudomonas aeruginosa ATCC 9027	11.1	12.3	13.1	0	0	15.6
Salmonella abony NTCC 6017	0	0	0	0	0	0
Candida albicans ATCC 10231	0	0	0	0	0	0
Saccharomyces cerevisiae ATCC 9763	0	0	0	0	0	0
Aspergillus brasiliensis ATCC 16404	0	0	0	0	0	0
Fusarium moniliforme	0	0	0	0	0	0

None of the tested compounds exhibited activity against *Salmonella abony*, nor against any of the tested fungal strains (*Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus brasiliensis*, *Fusarium moniliforme*).

4 Conclusions

Six novel compounds were successfully synthesized, and their physicochemical properties were determined. Structural characterization was performed using IR, ^{1}H NMR, ^{13}C NMR, and ^{13}C DEPT 135 spectroscopy. The synthesized compounds demonstrated good antibacterial activity against Gram-positive bacteria *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Bacillus cereus*, as well as the Gram-negative bacterium *Escherichia coli*. The compounds exhibited low or no activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. No antifungal activity was observed against the tested yeasts and molds.

The authors acknowledge the support of the Science Fund of the University of Ruse, Bulgaria (Project 2025/BRz-01). We are also grateful to Ms. Yoana Marinova (Sofia) for the valuable discussions.

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