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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF BIS-NAPHTHALIMIDE DERIVATIVES

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Abstract: *This work presents a new method for the synthesis of bis-naphthalimide derivatives based on the reaction of 2-(2-aminoethyl)-1H-benzo[de]isoquinoline-1,3(2H)-diones with 1H,3H-naphtho[1,8-cd]pyran-1,3-diones. The structures of the newly synthesized products were confirmed by melting points, R_f values, IR, ^{1}H NMR, ^{13}C NMR, and ^{13}C DEPT 135 spectroscopy. The antimicrobial activity of the obtained bis-naphthalimides was evaluated against Gram-positive and Gram-negative bacteria.*

Keywords: *bis-naphthalimides, IR, ^{1}H NMR, ^{13}C NMR, ^{13}C DEPT 135, antimicrobial activity.*

INTRODUCTION

Brana *et al.* (1997, 2003) were the first to report the synthesis of modified bis-naphthalimides and to investigate their cytotoxic activity, comparing it with that of the well-known antitumor agents elinafide (Fig. 1) and bisnafide (Fig. 2).

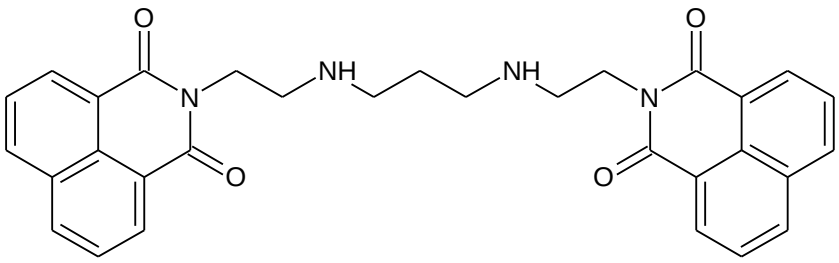


Fig. 1. Structure of elinafide

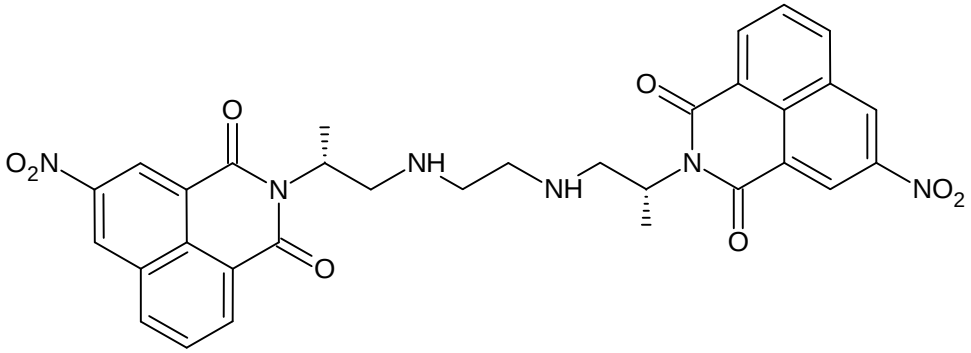
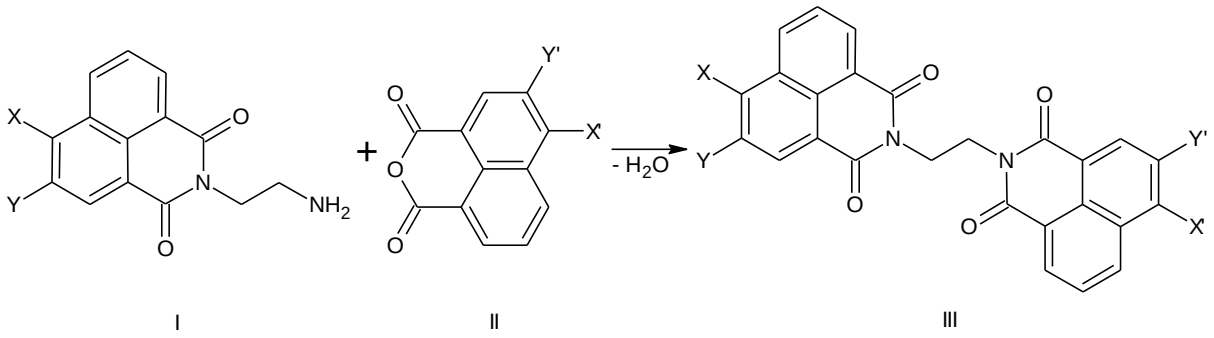


Fig. 2. Structure of bisnafide

We synthesized a new series of bis-naphthalimides from the corresponding mono-naphthalimides with the aim of developing new antimicrobial agents, as a continuation of our previous studies (Marinov *et al.*, in press). Using the methodology from the cited work, we obtained the corresponding 2-(2-aminoethyl)isoquinoline-1,3-dione derivatives (I), some of which have been previously reported by other authors (Palhares, 2015; Georgiev *et al.*, 2009; Bagale, 2011; Zhong *et al.*, 2021). The compounds I were subjected to condensation with various substituted naphthalene anhydrides (II), prepared according to our own modified methodologies (Kazhoka & Meyrovits, 1983; Rule & Thompson, 1937). The reactions were carried out in glacial acetic acid, resulting in the synthesis of the corresponding bis-naphthalimide derivatives (III), as illustrated in Fig. 3.



I II III

a) X = Y = X’ = Y’ = H; b) X = Br, Y = X’ = Y’ = H; c) Y = Br, X = X’ = Y’ = H;
d) X = X’ = Br, Y = Y’= H; e) Y = Y’= Br; X = X’ = H; f) X = Br; Y = NO_2, X’ = Y’ =H

Fig. 3. Synthetic scheme for bis-naphthalimides (IIIa–IIIf)

EXPERIMENTAL

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). The purity of the compounds was checked by thin layer chromatography on Kieselgel 60 F_{254}, 0.2 mm Merck plates, eluent system (vol. ratio): ethyl acetate : petroleum ether = 1 : 2.

Synthesis of compounds IIIa-IIIf

A mixture of the corresponding 1,8-naphthalic anhydride (0.01 mol) and the appropriate 2-(2-aminoethyl)isoquinoline-1,3-dione (0.01 mol) in glacial acetic acid (40 mL) was refluxed for 6 h. After cooling to room temperature, the resulting precipitate was filtered off, dried, and recrystallized from ethanol.

Determination of antimicrobial activity

The antimicrobial activity of the tested compounds was evaluated using the agar diffusion method. The following test microorganisms were employed: 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, and *Pseudomonas aeruginosa* ATCC 9027.

A 1% solution of each compound was prepared in dimethyl sulfoxide (DMSO). Tryptic soy agar (Merck) was used as the growth medium for bacteria. The medium was melted in a Koch apparatus, cooled to 48–50 °C, and inoculated with 1% of a previously prepared microbial suspension (≈10^7 CFU/mL, 0.5 McFarland standard). Sterile Petri dishes (Ø = 90 mm) were filled with 20 mL of inoculated medium and allowed to solidify. Wells (Ø = 8 mm) were cut in the agar and filled with 50 µL of the test solutions. After 30 min pre-diffusion at room temperature, the plates were incubated at 37 °C for 24 h (Hussein *et al.*, 2021).

Following incubation, inhibition zone diameters (mm) were measured using a digital caliper. Microbial sensitivity was interpreted as follows: ≤15 mm – low sensitivity; 15–25 mm – sensitive; >25 mm – highly sensitive. All experiments were performed in triplicate, and the antimicrobial effect of the solvent control was also evaluated. The reported values represent the arithmetic mean of three independent measurements.

RESULTS AND DISCUSSION

The physicochemical parameters of the obtained compounds are presented in Table 1.

Table 1. Physicochemical parameters of compounds IIIa-IIIf

№	Systematic name	Yield, %	M. p., °C	R_f*
IIIa	2,2'-(ethane-1,2-diyl)di(1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione)	73	288-289	0.36
IIIb	6-bromo-2-[2-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)ethyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione	84	291-292	0.32
IIIc	5-bromo-2-[2-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)ethyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione	69	275-276	0.35
IIId	2,2'-(ethane-1,2-diyl)bis(6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione)	75	297-298	0.28
IIIe	2,2'-(ethane-1,2-diyl)bis(5-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione)	79	266-267	0.30
IIIf	6-bromo-2-[2-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)ethyl]-5-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione	74	310-311	0.24

** Eluent system (vol. ratio): ethyl acetate : petroleum ether = 1 : 2*

A pronounced difference in melting points (> 100 °C) is observed between compounds Ia–Id and those of series IIIa–IIIg.

The IR spectroscopic data for compounds IIIa–IIIf are presented in Table 2, which clearly demonstrates the absence of absorption bands corresponding to the NH_2 group.

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

Compound	$\upsilon_{arom.}$	$\upsilon_{aliph.}$	$\upsilon_{C=O}$	υ_{C-N}	υ_{NO_2}
IIIa	3061	2886	1685, 1653	1350	
IIIb	3053	2851	1689, 1663	1347	
IIIc	3066	2850	1671, 1658	1351	
IIId	3058	2860	1689, 1664	1351	
IIIe	3069	2865	1691, 1672	1348	
IIIf	3062	2863	1690, 1668	1350	1532, 1361

The atom numbering used solely for the interpretation of the NMR spectral data (Table 3) is shown in Fig. 4.

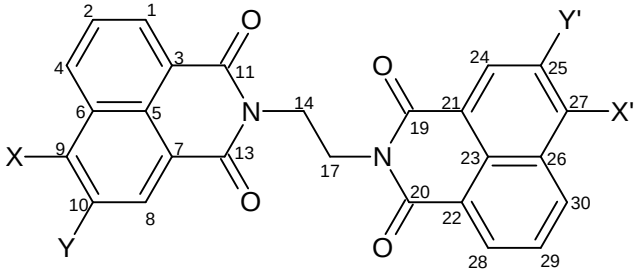


Fig. 4. General structural formula of compounds IIIa-IIIf

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

Atom No.	IIIa	IIIb	IIIc	IIIf	IIIa	IIIb	IIIc	IIId	IIIe	IIIf
	^{13}C NMR (DMSO-d_6, δ, ppm)*				^{1}H NMR (DMSO-d_6, δ, ppm)					
1	132.9	132.4	131.1	130.4	8.70	8.13	8.06	8.24	8.32	8.82
2	128.0	134.9	134.9	145.8	7.80	8.02	-	7.93	7.95	-
3	119.5	131.8	132.2	130.2	-	-	-	-	-	-
4	135.9	122.1	122.3	126.4	-	-	8.16	-	-	-
5	131.8	133.2	134.2	128.8	-	-	-	-	-	-
6	130.2	130.4	130.6	132.2	-	-	-	-	-	-
7	119.5	129.3	129.7	129.9	-	-	-	-	-	-
8	132.9	131.1	131.4	126.3	8.70	8.25	8.31	8.36	8.28	8.18
9	135.9	134.1	134.8	129.0	-	8.74	8.61	8.65	8.83	9.34
10	128.0	133.7	133.8	134.9	7.80	7.85	7.82	7.77	7.82	7.76
11	161.2	164.3	163.3	163.0	-	-	-	-	-	-
13	161.2	164.3	163.3	163.0	-	-	-	-	-	-
14	40.2	39.1	40.8	39.4	3.38	4.46	4.13	3.33/2.93	2.31	4.31
17	40.2	41.2	41.7	39.4	3.38	4.46	4.48	4.53/4.16	4.25	4.31
19	161.2	164.3	163.3	163.0	-	-	-	-	-	-
20	161.2	164.3	163.3	163.0	-	-	-	-	-	-
21	119.5	127.6	126.8	120.8	-	-	-	-	-	-
22	119.5	127.6	126.8	120.8	-	-	-	-	-	-
23	131.8	131.4	131.6	133.9	-	-	-	-	-	-
24	132.9	129.6	130.2	129.6	8.70	8.48	8.53	8.36	8.28	8.66
25	128.0	127.6	128.1	127.4	7.80	7.72	7.68	7.77	7.82	7.72
26	130.2	130.7	130.8	127.3	-	-	-	-	-	-
27	135.9	133.0	133.3	131.1	-	8.11	8.18	8.65	8.83	8.35
28	132.9	129.6	130.2	129.6	8.70	8.48	8.53	8.24	8.32	8.66
29	128.0	127.6	128.1	127.4	7.80	7.72	7.68	7.93	7.95	7.72
30	135.9	133.0	133.3	131.1	-	8.11	8.18	-	-	8.35

** These data are confirmed by ^{13}C DEPT 135 NMR spectroscopy.*

No ^{13}C NMR spectral data are provided in Table 3 for compounds IIId and IIIe due to their intense coloration.

The antimicrobial activity of the synthesized compounds was evaluated, and the results are presented in Table 4.

Table 4. Antimicrobial activity of compounds IIIa-IIIf

Test microorganism	**Inhibition zone diameter (mm)**					
	IIIa	**IIIb**	**IIIc**	**IIId**	**IIIe**	**IIIf**
Staphylococcus aureus ATCC 6538	12.5	14.1	14.5	15.4	16.2	16.8
Staphylococcus epidermidis ATCC 12228	15.2	15.9	16.3	16.7	15.5	18.1
Bacillus subtilis ATCC 6633	19.2	20.3	18.4	18.6	15.2	20.5
Bacillus cereus ATCC 10876	14.4	13.7	12.8	15.0	14.6	16.6
Pseudomonas aeruginosa ATCC 9027	12.0	13.1	13.0	15.3	14.2	14.7
Escherichia coli ATCC 8739	21.2	20.8	19.5	19.1	20.4	21.5

The Gram-negative bacterium *E. coli* was sensitive to compounds IIIa–IIIf. The largest inhibition zones were observed for compounds IIIa and IIIf (21.2 and 21.5 mm, respectively), followed by IIIb and IIIe (20.8 and 20.4 mm). Compounds IIIc and IIId exhibited slightly lower activity, with inhibition zones of 19.5 and 19.1 mm, respectively.

The Gram-positive bacterium *B. subtilis* was sensitive to compounds IIIa, IIIb, IIIc, IIId, and IIIf. Compounds IIIb and IIIf showed the highest activity against *B. subtilis* (20.3 and 20.5 mm), while IIIa exhibited moderate activity (19.2 mm). Compounds IIIc and IIId showed slightly lower inhibition zones (18.4 and 18.6 mm), and IIIe demonstrated the weakest activity (15.2 mm).

The Gram-positive bacterium *S. epidermidis* was sensitive to all tested compounds. The highest activity was observed for compound IIIf (18.1 mm). Compounds IIIc and IIId showed inhibition zones of 16.3 and 16.7 mm, respectively, while IIIa, IIIb, and IIIe exhibited lower activity, with inhibition zones ranging from 15.2 to 15.9 mm.

Compounds IIId, IIIe, and IIIf were active against the Gram-positive bacterium *S. aureus*, with inhibition zones of 15.4, 16.2, and 16.8 mm, respectively. Compounds IIIa, IIIb, and IIIc exhibited weak activity toward this test microorganism.

The Gram-positive bacterium *B. cereus* was sensitive to compounds IIId and IIIf, with inhibition zones of 15.0 and 16.6 mm, respectively. Compounds IIIa, IIIb, and IIIe exhibited weaker activity (14.4, 13.7, and 14.6 mm), whereas IIIc demonstrated the lowest activity (12.8 mm).

The results also revealed sensitivity of the Gram-negative bacterium *P. aeruginosa* to compound IIId, which produced a 15.3 mm inhibition zone. Lower activity was observed for IIIe and IIIf (14.2 and 14.7 mm), while compounds IIIa, IIIb, and IIIc showed weak inhibition (12.0, 13.1, and 13.0 mm).

CONCLUSION

Six new bis-naphthalimide derivatives were synthesized and characterized by physicochemical parameters, IR, and NMR spectroscopy. The results of the antimicrobial screening revealed that the compounds exhibited the highest activity against the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *Bacillus subtilis*.

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