Synthesis of 5-bromonaphthalimide derivatives with 3-aminocycloalkanespiro-5-hydantoins

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Abstract. The present work reports a study on the interaction of 5-bromo-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione with various 3-aminocycloalkanespiro-5-hydantoins, aimed at the development of new biologically active compounds. As a result of this condensation, seven new 5-bromonaphthalimide derivatives were synthesized, namely: 5-bromo-2-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione, 5-bromo-2-(6-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione, 5-bromo-2-(7-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione, 5-bromo-2-(8-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione, 5-bromo-2-(8-ethyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione, 5-bromo-2-(2,4-dioxo-8-propyl-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione, and 5-bromo-2-(2,4-dioxo-1,3-diazaspiro[4.7]dodecan-3-yl)benzo[*de*]isoquinoline-1,3-dione.The newly synthesized naphthalimides were characterized by physicochemical parameters as well as IR, ^{1}H NMR, and ^{13}C NMR spectral data. The antimicrobial activity of the described compounds was evaluated against Gram-positive bacteria, Gram-negative bacteria, yeasts, and molds. The tested products exhibited the strongest activity against the Gram-positive bacteria *Bacillus subtilis* and *Bacillus cereus*.

1 Introduction

Recent developments in the field of organic synthesis in the design of new effective agents have become increasingly significant due to the growing prevalence of various infectious and non-infectious diseases. It is well established that many 1,8-naphthalimide derivatives exhibit anticancer activity [1-7].

Various 1,8-naphthalimide derivatives have been investigated for their antimicrobial activity, including 1,8-naphthalimide aminothiazole hybrids [8], 4-sulfo-1,8-naphthalimides [9], and Cu(II) and Zn(II) metal complexes of polypropylene amine dendrimer modified with 4-bromo-1,8-naphthalimide [10].

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In our previous work, we reported the synthesis of a series of 1,8-naphthalimide derivatives of nalidixic acid and evaluated their antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, yeasts, and molds. Some of the compounds were found to exhibit activity against the tested Gram-positive and Gram-negative bacterial strains [11].

To the best of our knowledge, there are no published data on 5-bromonaphthalimides modified with cycloalkanespiro-5-hydantoins. Therefore, the aim of the present study is to describe a synthetic approach for the preparation of such derivatives, with the purpose of developing new biologically active compounds, particularly focusing 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). 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.

2.2 Synthesis of compounds IIIa–g (Fig. 1)

A mixture of 0.01 mol (2.78 g) of compound I and 0.01 mol of compounds IIa–g in 40 mL of glacial acetic acid was refluxed for 5 h. After cooling, the precipitate was filtered off and recrystallized from ethanol.

2.3 Determination of antimicrobial activity

The agar diffusion method was used to assess the antimicrobial activity of the compounds. Test organisms included: Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876), Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella abony* NTCC 6017), and yeast *Candida albicans* ATCC 10231. A 1% solution of each compound was prepared in DMSO. Tests were performed using Tryptic soy agar (Merck) for bacteria and Sabouraud dextrose agar (Merck) for yeasts. Media were melted in a Koch apparatus, cooled to 48–50 °C, and inoculated with 1% of previously prepared microbial suspensions (density ~10^7 cfu/mL, turbidity: 0.5 McFarland standard). In sterile Petri dishes (⌀ = 90 mm), 20 mL of the inoculated media was poured and allowed to solidify. Wells (⌀ = 8 mm) were punched into the agar, and 50 µL of each compound solution was applied. After a 30-minute pre-diffusion at room temperature, plates were incubated at 37 °C for 24 h (bacteria) and 28 °C for 48 h (yeasts) [12]. Zones of growth inhibition were measured in mm using a digital caliper: ≤15 mm – weak activity; 15–25 mm – moderate activity; ≥25 mm – strong activity. All experiments included solvent controls and were performed in triplicate.

3 Results and discussion

The starting compound, 5-bromo-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (synthesized *via* a modified procedure) [13], was reacted with 3-aminocycloalkanespiro-5-hydantoins [14, 15] in glacial acetic acid according to Fig. 1.

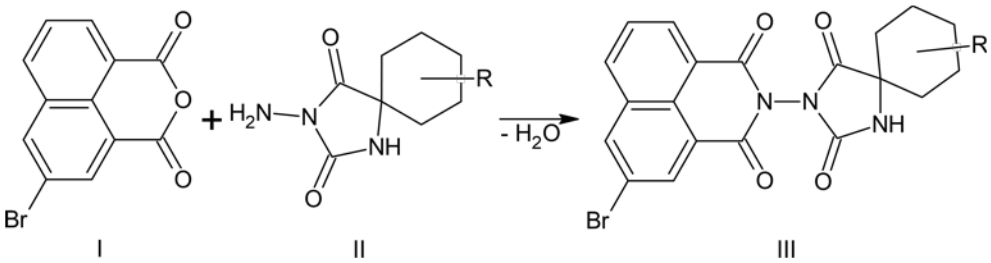

Fig. 1. Synthesis of compounds IIIa-g: a) R = H; b) R = 2-CH_3; c) R = 3-CH_3; d) R = 4-CH_3; e) R = 4-C_2H_5; f) R = 4-C_3H_7; g) cyclooctyl

The formation of the products was confirmed *via* melting points (m. p., °C), R_f (retention factor) values (Table 1), and IR/NMR spectral data (Tables 2-4).

Table 1. Physicochemical parameters of compounds IIIa-g

Compound	Systematic name	Yield, %	M. p., °C	R_f*
IIIa	5-bromo-2-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	91	247-248	0.53
IIIb	5-bromo-2-(6-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	83	263-264	0.51
IIIc	5-bromo-2-(7-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	85	295-296	0.55
IIId	5-bromo-2-(8-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	94	272-273	0.49
IIIe	5-bromo-2-(8-ethyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	96	263-264	0.46
IIIf	5-bromo-2-(2,4-dioxo-8-propyl-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	92	288-289	0.50
IIIg	5-bromo-2-(2,4-dioxo-1,3-diazaspiro[4.7]dodecan-3-yl)benzo[*de*]isoquinoline-1,3-dione	97	256-257	0.43

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

Compared to the starting 3-aminocycloalkanespiro-5-hydantoins (m. p.: 166-200°C), the synthesized naphthalimide derivatives exhibited significantly higher melting points (247-296°C).

The IR spectra indicate the disappearance of the characteristic vibrations of the NH2 group [14, 15] in the region between 3200 and 3320 cm-1, accompanied by the appearance of new absorption bands corresponding to NH group vibrations, observed in the region between 3250 and 3335 cm-1. The aromatic and aliphatic vibrations are observed at 3058–

3072 cm-1 and 2918–2943/2855–2876 cm-1, respectively. Evidence for the presence of the two carbonyl groups of the hydantoin ring is provided by the absorption bands at 1796–1825 cm-1 and 1757–1775 cm-1, corresponding to the C^2=O and C^4=O vibrations, respectively.

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

Compound	ϑ_{NH}	ϑ_{arom}	ϑ_{aliph}	$\vartheta_{C^2=O}$	$\vartheta_{C^4=O}$
IIIa	3338	3061	2935, 2860	1825	1769
IIIb	3341	3058	2943, 2855	1821	1760
IIIc	3325	3065	2918, 2859	1815	1775
IIId	3345	3072	2920, 2862	1811	1762
IIIe	3348	3063	2931, 2865	1808	1769
IIIf	3342	3059	2936, 2871	1803	1771
IIIg	3345	3066	2929, 2876	1796	1757

Table 3 compares the ^{1}H NMR spectral data of the starting 3-aminocycloalkanespiro-5-hydantoins (IIa–g) with those of the synthesized naphthalimides (IIIa–g). The signals corresponding to the NH_2 group in the region of 3.90 to 4.62 ppm [14, 15] disappear, while the signals for the NH group in the hydantoin core remain slightly shifted, appearing between 9.31 and 11.32 ppm. This confirms the successful condensation between 5-bromo-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (I) and the corresponding 3-aminocycloalkanespiro-5-hydantoins (IIa–g**)**.

Table 3. ^{1}H NMR (DMSO-d_6, δ, ppm) spectral data of compounds IIIa-g

Compound	NH and NH_2	Ref.	Compound	NH
IIa	8.50 (1H, s) and 4.60 (2H, s)	[14]	**IIIa**	9.31 (1H, s)
IIb	8.25 (1H, s) and 4.70 (2H, s)	[15]	**IIIb**	10.50 (1H, s)
IIc	8.12 (1H, s) and 4.80 (2H, s)	[14]	**IIIc**	11.32 (1H, s)
IId	8.18 (1H, s) and 5.01 (2H, s)	[15]	**IIId**	9.50 (1H, s)
IIe	8.28 (1H, s) and 5.02 (2H, s)	[15]	**IIIe**	10.65 (1H, s)
IIf	8.45 (1H, s) and 5.00 (2H, s)	[15]	**IIIf**	11.12 (1H, s)
IIg	8.60 (1H, s) and 3.90 (2H, s)	[14]	**IIIg**	10.35 (1H, s)

Table 4. ^{13}C NMR (DMSO-d_6, δ, ppm) spectral data of compounds IIIa-g*

Atom No.	Compound						
	IIIa	**IIIb**	**IIIc**	**IIId**	**IIIe**	**IIIf**	**IIIg**
1	120.8	120.6	120.8	125.8	123.4	121.1	120.5
2	130.2	130.1	130.1	130.4	130.2	130.1	130.1
3	132.1	132.9	132.9	131.8	132.9	132.2	132.5
4	120.8	119.8	119.5	120.8	120.2	119.9	120.2
5	126.4	128.0	128.1	130.1	128.0	128.1	129.6
6	133.2	132.9	132.1	132.9	132.6	132.9	132.9
7	119.5	119.5	120.0	119.5	119.5	119.5	119.5
8	138.2	137.2	138.2	136.8	137.3	137.3	137.4
9	136.8	135.9	136.8	135.8	136.8	135.9	135.9
10	121.0	121.8	121.1	128.2	121.1	121.1	121.1
11	160.0	160.0	159.9	160.0	160.0	160.0	160.5
12	160.9	160.9	160.9	160.9	160.9	160.9	160.9
13	161.2	161.1	161.1	161.2	161.1	161.1	161.1
14	174.6	173.5	173.6	174.5	174.5	173.9	174.1
15	64.1	66.8	62.3	61.5	64.2	64.5	63.6
16	37.6	34.6	41.8	33.8	34.5	35.3	32.7
17	22.4	23.3	27.3	29.2	30.2	32.4	23.3
18	29.2	30.5	33.4	31.0	31.5	33.1	28.6
19	22.4	25.3	33.3	29.9	30.2	32.4	28.1
20	37.6	39.9	41.8	33.8	34.5	35.3	28.6
21	-	15.8	22.5	22.0	21.7	25.5	23.3
22	-	-	-	-	28.6	27.8	32.7
23	-	-	-	-	-	20.6	-

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

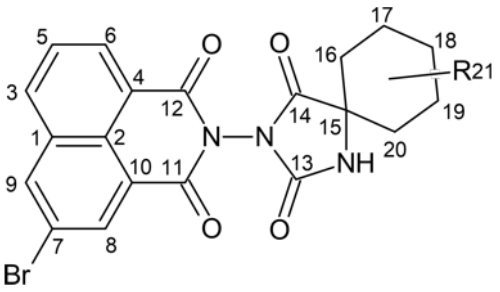

Fig. 2. General structural formula of compounds IIIa-g

The numbering of the carbon atoms in the structural formula of naphthalimides IIIa-g (Fig. 2) is intended solely for the interpretation of their ^{13}C NMR spectra (Table 4).

The ^{13}C NMR spectral data presented in Table 4 are in complete agreement with the proposed structures of the synthesized 5-bromonaphthalimides modified with cycloalkanespiro-5-hydantoins.

The signals for the imide and hydantoin carbonyl carbons appear at 159.9–160.5 ppm (C^{11})**,** 160.9 ppm (C^{12})**,** 161.1 ppm and 161.2 ppm (C^{13}), and 173.5–174.6 ppm (C^{14}), respectively. In the region 119.5–138.2 ppm, ten signals are observed, corresponding to the aromatic carbons (C^{1}–C^{10}) of the naphthalene skeleton. The signals for the cycloalkyl carbons (C^{16}–C^{20}) appear in the region 22.4–41.8 ppm, while the resonance at 61.5–66.8 ppm is assigned to the spiro-carbon (C^{15}).

Table 5. Antimicrobial activity of compounds IIIa-g

Test microorganism	**Inhibition zone diameter (mm)**						
	IIIa	**IIIb**	**IIIc**	**IIId**	**IIIe**	**IIIf**	**IIIg**
Staphylococcus aureus ATCC 6538	12.5	10.8	13.3	12.1	13.5	13.9	11.1
Staphylococcus epidermidis ATCC 12228	13.1	12.6	14.2	13.4	13.9	14.4	13.3
Bacillus subtilis ATCC 6633	16.4	15.4	17.1	16.2	15.8	16.1	16.5
Bacillus cereus ATCC 10876	18.2	17.9	18.4	17.1	18.8	18.9	18.2
Pseudomonas aeruginosa ATCC 9027	10.8	10.1	10.5	10.9	11.1	12.2	11.2
Escherichia coli ATCC 8739	14.4	13.7	12.6	14.5	14.7	15.3	14.3
Salmonella abony NTCC 6017	11.6	10.4	12.5	11.7	12.2	12.5	12.1
Candida albicans ATCC 10231	13.7	12.6	13.3	14.1	13.6	13.8	13.5

The antimicrobial activity of compounds IIIa–g was evaluated against Gram-positive bacteria, Gram-negative bacteria, and yeast using the agar diffusion method. The measured inhibition zone diameters (mm) are summarized in Table 5.

Analysis of the experimental data revealed that the tested compounds exhibit antimicrobial activity, with higher efficacy against Gram-positive bacteria, particularly

Bacillus cereus ATCC 10876 and *Bacillus subtilis* ATCC 6633, showing inhibition zones in the ranges of 17.1–18.9 mm and 15.4–17.1 mm, respectively.

It should be noted that compounds IIIc, IIIe, and IIIf demonstrated the highest overall antibacterial activity, which was most pronounced against *Bacillus cereus* (18.4–18.9 mm) and Bacillus *subtilis* (15.8–17.1 mm). The inhibition zones recorded against *Staphylococcus aureus* and *Staphylococcus epidermidis* were in the range of 10.8–14.4 mm.

The tested Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, Salmonella *abony*) were less sensitive to the investigated compounds, with inhibition zones between 10.1 and 15.3 mm.

The synthesized products also exhibited lower activity against the pathogenic yeast *Candida albicans*, with inhibition zones ranging from 12.6 to 14.1 mm; the strongest antifungal effect (14.1 mm) was observed for compound IIId.

4 Conclusions

Seven new derivatives of 5-bromonaphthalimide with 3-aminocycloalkanespiro-5-hydantoins were synthesized. Their structures were confirmed using IR and NMR spectroscopy, and some physicochemical parameters were determined.

The compounds' antimicrobial activities were evaluated against various Gram-positive and Gram-negative bacteria and yeast. It was found that the compounds exhibit activity against the tested microorganisms, with the strongest effect observed against the Gram-positive bacteria *Bacillus subtilis* and *Bacillus cereus*. The obtained results clearly indicate that the synthesized products possess promising antibacterial potential, particularly against Gram-positive strains. These findings highlight the need for further studies on related compounds aimed at the discovery and development of new and effective antimicrobial agents.

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