Synthesis and antimicrobial activity of 5-(2-aminoethylamino)-2-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-diones

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Abstract. This article presents a method for the synthesis of novel 5-(2-aminoethylamino)-2-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-diones, based on the reaction of various 5-bromo-2-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-diones with ethane-1,2-diamine. The resulting naphthalimide derivatives were characterized using physicochemical methods, IR, ^{1}H NMR, and ^{13}C NMR spectroscopy. Furthermore, their antimicrobial activity was evaluated against both Gram-positive and Gram-negative bacteria, as well as yeasts and molds.

1 Introduction

The interest in studying 1,8-naphthalimide derivatives arises from their broad spectrum of biological activities. For instance, numerous representatives of this class of compounds are well known for their antimicrobial properties [1-5]. Additionally, it should be noted that similar investigations have also been carried out on various spirohydantoin derivatives [6, 7].

For the first time, an attempt was made to substitute the bromine atom of 5-bromo-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione with ethane-1,2-diamine simultaneously with condensation with 3-aminocycloalkanespiro-5-hydantoins. While substitution of the bromine atom in the sixth position of 1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione with alkylamines has been reported [8], the synthesis of derivatives with improved biological activity is novel.

Since no data were found regarding 5-(2-aminoethylamino)-2-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-diones, the aim of the present study was to develop a method for obtaining a series of such compounds in the search for new antimicrobial agents.

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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 Va–g

A mixture of compounds IVa–g (0.01 mol) in an excess of pure ethane-1,2-diamine (10 mL) was stirred overnight at room temperature, yielding a dark orange liquid. The crude product was slowly added to distilled water (50 mL) and stirred for 2 hours. The precipitate was filtered and washed repeatedly with distilled water to obtain a yellow amorphous solid.

2.3 Synthesis of compound VI

A mixture of compound Vd (0.01 mol) and 4-methoxybenzaldehyde (0.01 mol) in 20 mL methanol with a few drops of glacial acetic acid was heated in a water bath for 6 hours. After cooling, the product was filtered and recrystallized from ethanol.

2.4 Determination of antimicrobial activity

Antimicrobial activity was assessed by the agar diffusion method against the following test microorganisms: 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), yeasts (*Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9763), and molds (*Aspergillus brasiliensis* ATCC 16404, *Fusarium moniliforme*) [9]. The tests were carried out using 1% solutions of the synthesized compounds in DMSO. Zones of growth inhibition were measured in mm using a digital caliper. The criteria for evaluating antimicrobial activity were as follows: ≤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

5-Bromo-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione was synthesized *via* a modified procedure [10], followed by condensation with 3-aminospirohydantoins [11–15] (Fig. 1-3). An attempt was made to obtain a Schiff base of compound Vd with 4-methoxybenzaldehyde according to Fig. 4.

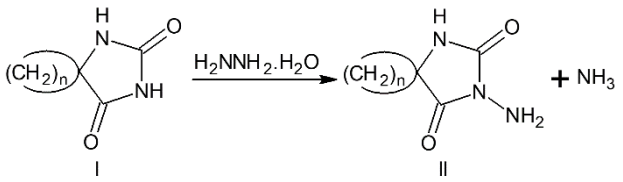


Fig. 1. Synthesis of compounds II.

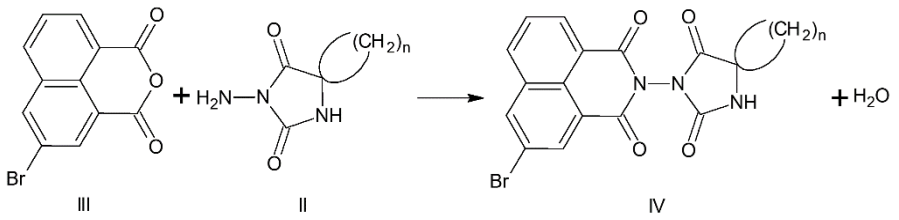
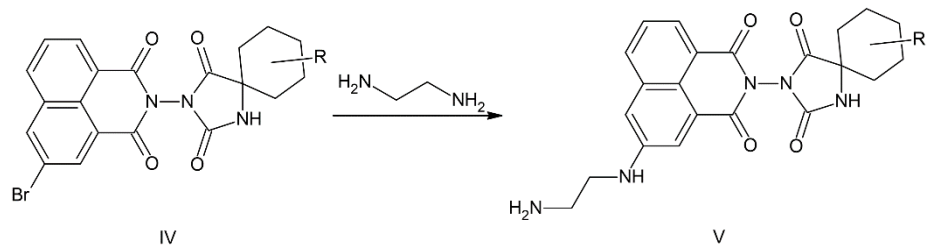


Fig. 2. Synthesis of compounds IV



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

Fig. 3. Synthesis of compounds V

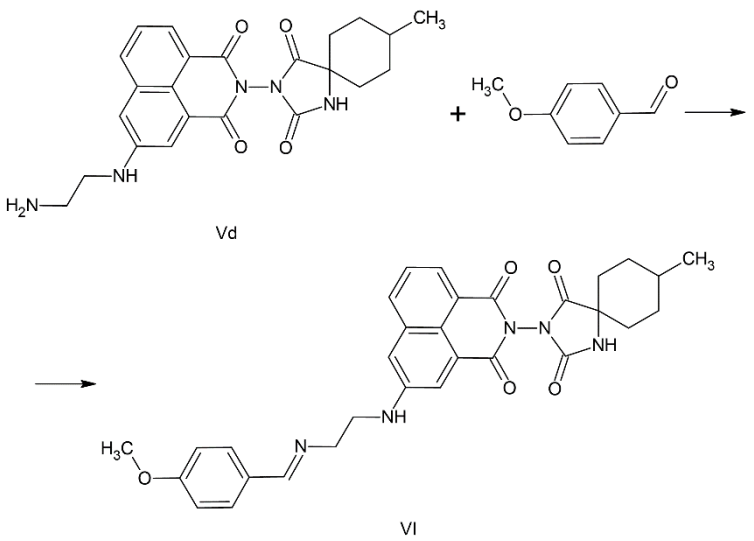


Fig. 4. Synthesis of compound VI

The physicochemical characteristics of products Va–g and VI are presented in Table 1. It should be noted that the melting points of compounds Va–g are approximately 100 °C lower than those of IVa–g.

Table 1. Physicochemical parameters of compounds Va-g and VI

№	Systematic name	Yield, %	M. p., °C	R_f
Va	5-(2-aminoethylamino)-2-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	94	156-157	0.45
Vb	5-(2-aminoethylamino)-2-(6-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	98	167-168	0.42
Vc	5-(2-aminoethylamino)-2-(7-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	94	136-137	0.46
Vd	5-(2-aminoethylamino)-2-(8-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	91	160-161	0.40
Ve	5-(2-aminoethylamino)-2-(8-ethyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	88	138-139	0.35
Vf	5-(2-aminoethylamino)-2-(2,4-dioxo-8-propyl-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	72	177-178	0.39
Vg	5-(2-aminoethylamino)-2-(2,4-dioxo-1,3-diazaspiro[4.7]dodecan-3-yl)benzo[*de*]isoquinoline-1,3-dione	95	143-144	0.32
VI	5-[2-[(*E*)-(4-methoxyphenyl)methyleneamino]ethylamino]-2-(8-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione	86	195-196	0.34

In the IR spectra (Table 2), absorption bands corresponding to NH_2 group vibrations, absent in IVa–g, are observed.

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

№	υ_{NH_2}	υ_{NH}	$\upsilon_{C=O}$	$\upsilon_{arom.}$	$\upsilon_{aliph.}$	υ_{NH}	υ_{C-N}
Va	3382, 3271	3186	1796, 1754, 1725, 1688	3063	2912	1623	1251
Vb	3385, 3274	3191	1801, 1772, 1730, 1691	3078	2925, 2886	1625	1254
Vc	3389, 3278	3195	1803, 1773, 1732, 1694	3072	2932, 2885	1625	1253
Vd	3393, 3282	3189	1800, 1775, 1728, 1692	3065	2935, 2881	1625	1254
Ve	3379, 3268	3191	1795, 1750, 1716, 1689	3064	2938, 2882	1625	1253
Vf	3375, 3264	3190	1793, 1748, 1712, 1685	3068	2941, 2886	1625	1254
Vg	3370, 3259	3189	1790, 1746, 1706, 1677	3065	2944, 2883	1625	1254
Vh		3205	1779, 1732, 1695, 1675	3071	2951, 2882	1622	1251
VI		3221, 3165	1730, 1711, 1690, 1675				

In the ^{1}H NMR spectra (Table 3), signals corresponding to NH_2, NH, and CH_2 groups are present.

The general structural formula of compounds Va–g is presented in Fig. 5, with the clarification that the indicated atom numbering is provided solely for the interpretation of the ^{13}C NMR spectra of the products. The ^{13}C NMR spectral data for compounds Va-g are provided in Table 4. For compound VI, ^{13}C NMR signals are observed at: 163.5, 162.3, 158.9, 157.2 ppm (C=O); 160.7 ppm (CH=N); 62.8 ppm (spiro-carbon).

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

№	NH and NH_2
Va	8.09 (s, 2H, NH_2), 6.74 (1H, s), 4.48 (1H, s), 1.71-2.09 (m, 10H)
Vb	8.67 (s, 2H, NH_2), 6.75 (1H, s), 4.47 (1H, s), 2.38-2.83 (m, 8H), 1.21 (s, 3H)
Vc	8.63 (s, 2H, NH_2), 6.74 (1H, s), 4.47 (1H, s), 2.37-2.83 (m, 8H), 1.65 (s, 3H)
Vd	8.68 (s, 2H, NH_2), 6.74 (1H, s), 4.48 (1H, s), 2.52-2.82 (m, 8H), 1.21 (s, 3H)
Ve	8.68 (s, 2H, NH_2), 6.74 (1H, s), 4.45 (1H, s), 2.51-2.82 (m, 8H), 1.37 (s, 2H), 0.99 (s, 3H)
Vf	8.69 (s, 2H, NH_2), 6.76 (1H, s), 4.48 (1H, s), 2.51-2.83 (m, 8H), 1.19 (s, 2H), 1.43 (s, 2H), 0.98 (s, 3H)
Vg	8.85 (s, 2H, NH_2), 6.75 (1H, s), 4.47 (1H, s), 2.38-2.83 (m, 14H)
Vh	8.32 (s, CH), 6.97 (1H, s), 4.49 (1H, s), 1.99-2.65 (m, 8H), 1.07 (s, 3H)
VI	5.20 (s, 1H, NH), 4.25 (s, 1H, NH), 1.60-2.33 (m, 8H, CH_2), 1.06 (s, 3H, CH_3)

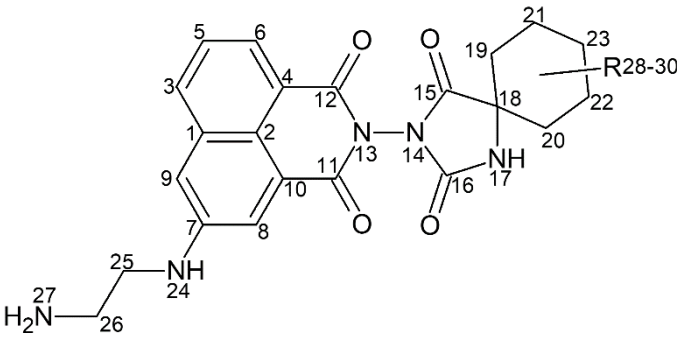


Fig. 5. Compounds Va-g

The spectral data presented in Tables 2–4 unambiguously confirm the proposed structures of the synthesized compounds.

The antimicrobial activity of products Va–g was evaluated (Table 5) and found to be significantly higher than that of IVa–g.

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

№	Va	Vb	Vc	Vd	Ve	Vf	Vg
1	135.9	135.9	135.9	134.6	134.6	134.6	134.6
2	127.8	127.6	125.8	125.8	127.8	127.8	127.8
3	134.6	134.8	134.6	131.7	131.5	131.7	131.7
4	131.7	131.7	133.6	128.2	128.2	131.0	131.0
5	132.7	132.7	133.0	131.2	131.0	131.3	131.3
6	131.3	128.7	127.8	127.8	128.8	128.8	128.8
7	145.2	145.1	145.2	145.2	145.2	145.2	145.2
8	127.6	122.6	127.6	127.6	120.1	127.6	127.6
9	122.6	122.2	122.6	122.6	117.8	122.6	122.6
10	128.8	127.8	128.7	126.6	126.6	126.6	126.8
11	157.5	157.5	157.6	157.5	157.5	156.3	157.5
12	159.1	159.0	159.0	159.0	159.1	159.0	159.0
15	164.1	164.2	164.1	164.1	163.5	175.6	164.1
16	161.4	164.0	164.0	161.4	161.4	164.1	161.0
18	62.5	66.8	64.1	62.8	62.8	60.0	60.0
19	32.3	34.6	41.9	29.2	29.9	29.9	34.0
20	32.3	30.3	32.7	29.2	29.9	22.9	23.3
21	22.8	30.2	27.5	30.5	27.8	28.3	23.3
22	22.8	25.6	20.5	30.5	27.8	28.3	28.6
23	25.8	25.4	22.6	31.6	37.8	35.6	28.1
25	43.2	43.1	43.2	46.8	46.9	46.7	46.8
26	40.2	40.1	40.2	40.5	40.6	40.5	40.5
28		15.3	22.6	21.9	12.3	20.6	
29						14.4	

** Data confirmed by ^{13}C DEPT 135 spectroscopy.*

The results presented in Table 5 indicate that compounds Va–g exhibit moderate activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella abony*, and *Candida albicans*, and strong activity against *Pseudomonas aeruginosa*.

Compound VI was inactive against the tested microorganisms.

Table 5. Antimicrobial activity of compounds Va-g and VI

Test microorganism	Inhibition zone diameter (mm)							
	Va	**Vb**	**Vc**	**Vd**	**Ve**	**Vf**	**Vg**	**VI**
Staphylococcus aureus ATCC 6538	19.8	20.6	19.4	21.5	21.7	21.3	20.2	0
Staphylococcus epidermidis ATCC 12228	21.3	20.8	22.2	22.3	22.4	22.1	21.1	0
Bacillus subtilis ATCC 6633	20.5	22.4	23.5	22.9	23.3	23.5	21.4	0
Bacillus cereus ATCC 10876	22.6	21.5	20.8	21.8	22.2	22.7	21.2	0
Pseudomonas aeruginosa ATCC 9027	24.4	25.6	24.6	25.3	25.1	25.6	23.7	0
Escherichia coli ATCC 8739	20.8	21.4	22.3	22.6	21.8	22.4	21.3	0
Salmonella abony NTCC 6017	18.2	19.3	20.1	20.8	20.3	20.2	19.9	0
Candida albicans ATCC 10231	21.4	21.8	21.7	21.5	21.5	22.1	20.4	0

4 Conclusions

A series of 5-(2-aminoethylamino)-2-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-diones (Va–g), as well as the Schiff base 5-[2-[(*E*)-(4-methoxyphenyl)methyleneamino]ethylamino]-2-(8-methyl-2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)benzo[*de*]isoquinoline-1,3-dione (VI), were synthesized and structurally characterized.

Antimicrobial studies demonstrated that, unlike compound VI, compounds Va–g exhibit activity against the tested Gram-positive and Gram-negative bacteria, yeasts, and molds. It was found that compounds Va–g are most active against the Gram-negative bacterium *Pseudomonas aeruginosa*.

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