SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 1,8-NAPHTHALIMIDE DERIVATIVES OF NALIDIXIC ACID

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ABSTRACT

Various naphthalimides are synthesized and their biological activity is studied. The target compounds are prepared by the interaction of different substituted 2-amino-1H-benzo[de]isoquinoline-1,3(2H)-diones with nalidixic acid (1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid). The structures of all synthesized products are verified via their physicochemical parameters, an elemental analysis, IR, ^{1}H and ^{13}C NMR spectroscopy. The antimicrobial activity of the compounds obtained is determined against Gram-positive bacteria Staphylococcus aureus and Bacillus subtilis, Gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa and Salmonella abony, the yeasts Candida albicans and Saccharomyces cerevisiae and the molds Penicillium chrysogenum and Aspergillus niger. It is found that N-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide, N-(6-1H-indol-3-yl-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide and N-(6-piperidin-1-yl-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide are active against the Gram-positive and Gram-negative bacteria tested.

Keywords: 2-amino-1H-benzo[de]isoquinoline-1,3(2H)-diones; 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid; Gram-positive bacteria; Gram-negative bacteria.

INTRODUCTION

Various biologically active 1,8-naphthalimide derivatives are known as antitumor agents [1 - 16], enzyme inhibitors [17], antivirals [18 - 22], analgesic and antirheumatic drugs [23], antimicrobials [24 - 28].

A general method of 1,8-naphthalimides synthesis refers to the interaction of the corresponding 1,8-naphthalic anhydrides with primary amines [1]. The reaction and the atoms numbering in the final products are shown in Scheme 1.

Nalidixic acid (Fig. 1) corresponds to the first generation of 4-quinolones, i.e. antibacterial drugs which are derivatives of 4-quinolone-3-carboxylic acid. It is used in the treatment of urinary infections, as it manifests extremely successful bacteriostatic action, strongly expressed against some Gram-positive microorganisms and limited to certain Gram-negative microorganisms [29 - 31]. The 4-quinolones (such as oxolinic acid, pipedimic acid and cinoxacin) developed later are representatives of the second generation of quinolones. They do not have significant advantages and are rarely used in the treatment of such infections [29 - 31]. The introduction of fluorine and piperidine substituent,

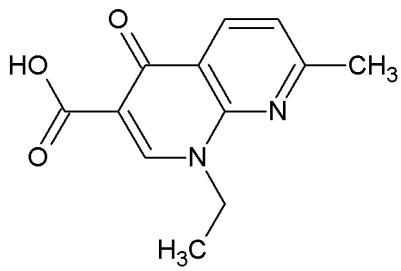


Fig. 1. Nalidixic acid (INN) /1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid/.

respectively, at positions 6 and 7 of the quinolone ring, brings about an increase of the antibacterial activity and a broadening of the action spectrum. Thus fluoroquinolones, third generation drugs, have been obtained. They are distinguished by their bactericidal action against Gram-negative microorganisms comparable to that of the third generational cephalosporins and aminoglycosides. Like macrolides, they have a good intracellular distribution in case of an oral route and are well tolerated. Furthermore, resistance to them is rarely developed [29 - 31].

The synthesis of *N*-(2,4-dioxo-1,3-diazaspiro[4.5] decan-3-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (Fig. 2) based on the reaction of 3-aminocyclohexanespiro-5-hydantoin with nalidixic acid is recently presented [32]. It is found that this compound exhibits a certain antimicrobial activity against the tested Gram-positive and Gram-negative bacteria.

The data presented above determines the interest in the study of the effect that would have resulted from the combination of the biological action of naphthalimide with that of biologically active acids such as nalidixic acid. Therefore, the aim of this study is the synthesis of such compounds whose bacteriostatic effect influences both Gram-positive and Gram-negative microorganisms.

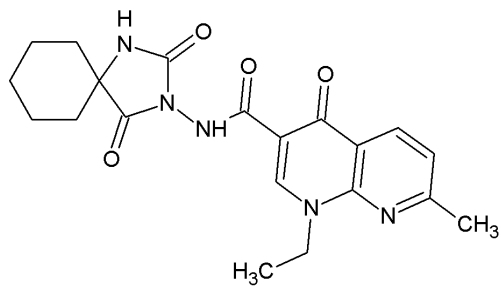


Fig. 2. *N*-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide.

EXPERIMENTAL

General

All chemicals used were purchased from Merck and Sigma-Aldrich. The melting points were determined by a SMP-10 digital melting point apparatus. The purity of the compounds was checked by thin layer chromatography on Kieselgel 60 F_{254}, 0.2 mm Merck plates using an eluent system (vol. ratio) of ethyl acetate : petroleum ether = 1 : 2. The elemental analysis data was obtained with an automatic analyzer Carlo Erba 1106. The IR spectra were taken on Perkin-Elmer FTIR-1600 spectrometer using KBr discs. The NMR spectra were recorded on a Bruker DRX-250 spectrometer operating at 250.13 MHz 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 $CDCl_3$ solutions were carried out at an ambient temperature (300 K). The typical ^{1}H NMR spectra conditions referred to: a pulse width of 30º, 1 s relaxation delay, 16K time domain points, zero-filled to 64K, a hard pulse with 90º pulse width of 11.8 μs. Those referring to ^{13}C NMR spectra included a pulse width 30º, 1 s relaxation delay, 16K time domain points, zero-filled to 32K, a hard pulse with 90º pulse width of 6.4 μs at a power level of 3 dB below the maximum output.

Synthesis of 6-substituted 2-amino-1*H*-benzo[de] isoquinoline-1,3(2*H*)-diones (7a - 7h, Scheme 4)

A mixture of 0.035 mol of the respective 6-substituted 1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-diones (6a - 6h) and 1.94 ml of 95 % hydrazine hydrate was refluxed for 4 h in 50 ml of acetic acid. After cooling to a room temperature, the solution formed was poured into 300 ml of cold water. The product obtained was filtered off, dispersed in water and alkalized with ammonium hydroxide till pH 8 - 9. The resulting solution was extracted with methylene chloride (3 x 50 ml). The organic layer was dried over magnesium sulfate and then filtered off. The solvent was evaporated under vacuum and the products obtained (7a - 7h) were recrystallized from benzene.

Synthesis of 6-substituted *N*-(1,3-dioxo-1*H*-benzo[de] isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamides (9a - 9h, Scheme 5)

A mixture of 0.01 mol of compounds 7a-7h, 2.32 g (0.01 mol) of nalidixic acid (8) and 2.06 g (0.01

mol) of *N,N'*-dicyclohexylcarbodiimide (DDC) were stirred for an hour at a room temperature in 50 ml of tetrahydrofuran. The resulting solution was left overnight, then *N,N'*-dicyclohexylcarbamide formed was filtered off. Glacial acetic acid (1 ml) was added to the filtrate, and the additional quantity of dicyclohexylurea formed was filtered. The solvent was evaporated under vacuum and the resulting products (9a-9h) were recrystallized from ethanol.

Antimicrobial assay

The microbial cultures were purchased from National Bank of Industrial Microorganisms and Cell Cultures (NBIMCC), Sofia. The antimicrobial effect of the synthesized compounds 9a-9h against Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633, Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NTCC 6017, the yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763 and the molds *Penicillium chrysogenum* and *Aspergillus niger* was studied. The agar well diffusion method [33] was the technique used for this investigation. The agar media chosen referred to Soy-casein agar (Scharlau) for the tests with bacteria and Sabouraud dextrose agar (Scharlau) for yeast and molds. The wells were filled with ethylene glycol solutions (50 µl) of the compounds synthesized. Following a 30 min stay at a room temperature, the Petri dishes were placed in a thermostat at 37°C for 24 h for bacteria, at 28°C for 24 h for yeasts and 72 h for molds. After cultivation, the diameter of inhibition growth zones around the wells was measured in mm, and the results obtained were evaluated as follows: up to 15 mm - the microbial culture sensitivity was week, 15 - 20 mm - it was good and higher than 25 mm - the microbial culture was very sensitive to the given synthetic compound at the tested concentration (5 mg/ml). Pure ethylene glycol (50 µl) was used as a control sample. The data on antimicrobial activity was evaluated as an arithmetic average of three measurements.

RESULTS AND DISCUSSION

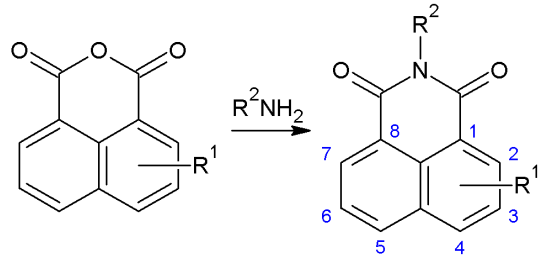
The initial substituted 1,8-naphthalic anhydrides are prepared by applying the methods presented in Schemes 2 and 3, respectively.

6-Nitronaphthalic anhydride (6-nitro-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione, 3) is synthesized by the reaction of acenaphthene (1,2-dihydroacenaphthylene, 1) with HNO_3 to form 5-nitroacenaphthene (5-nitro-1,2-dihydroacenaphthylene, 2) and subsequent treated with $K_2Cr_2O_7$ in CH_3COOH (Scheme 2).

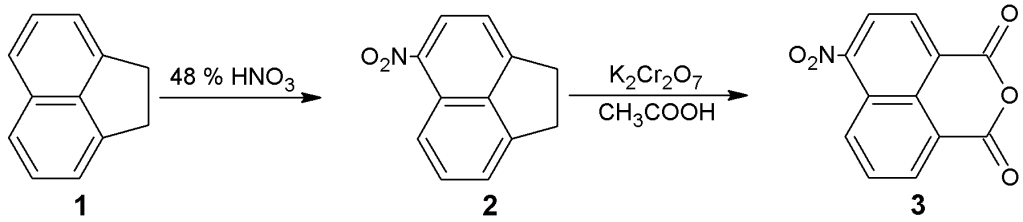
The method for the synthesis of 6-bromonaphthalic anhydride (6-bromo-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione, 5) is based on the interaction of acenaphthene (1,2-dihydroacenaphthylene, 1) with CH_3OH and Br_2 at 0°C and further treatment of the resulting 5-bromo-acenaphthene (5-bromo-1,2-dihydroacenaphthylene, 4) with $K_2Cr_2O_7$, CH_3COONa and CH_3COOH (Scheme 3).

In the last part of the synthesis, the substitution of Br is carried out by refluxing equimolar amounts of the reactants in *N,N*-Dimethylformamide (DMF). This results in the successful preparation of 6-substituted naphthalic anhydrides (6) with pyrrolidine, piperidine, morpholine, phenothiazine and indole substituent.

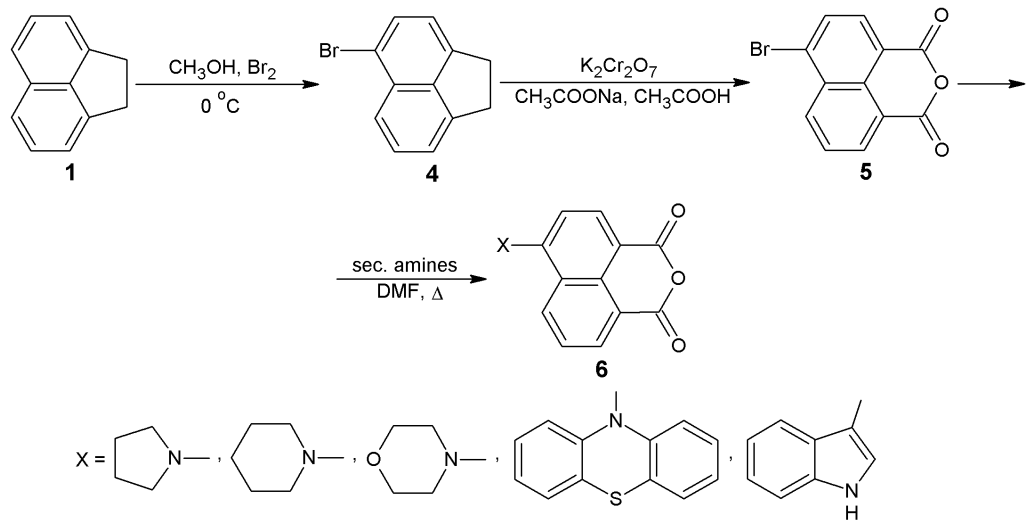
Later in the text, as a general structural formula, of both the unsubstituted 1,8-naphthalic anhydride and



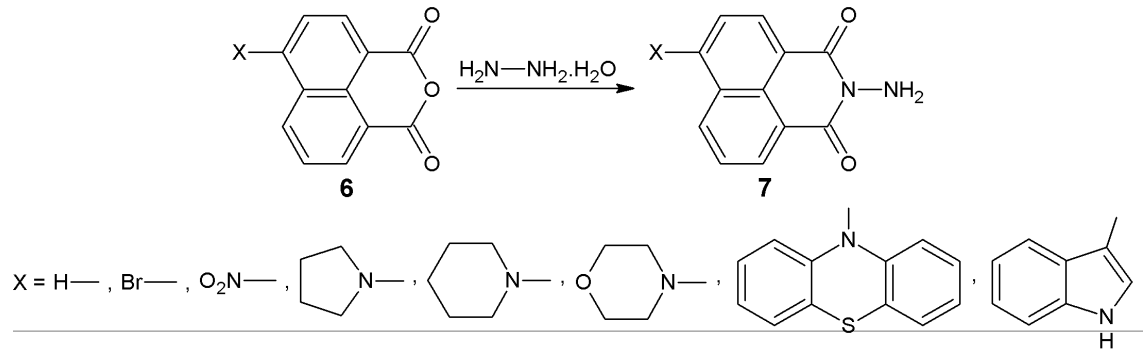
Scheme 1. Synthesis and the atoms numbering of 1,8-naphthalimides.



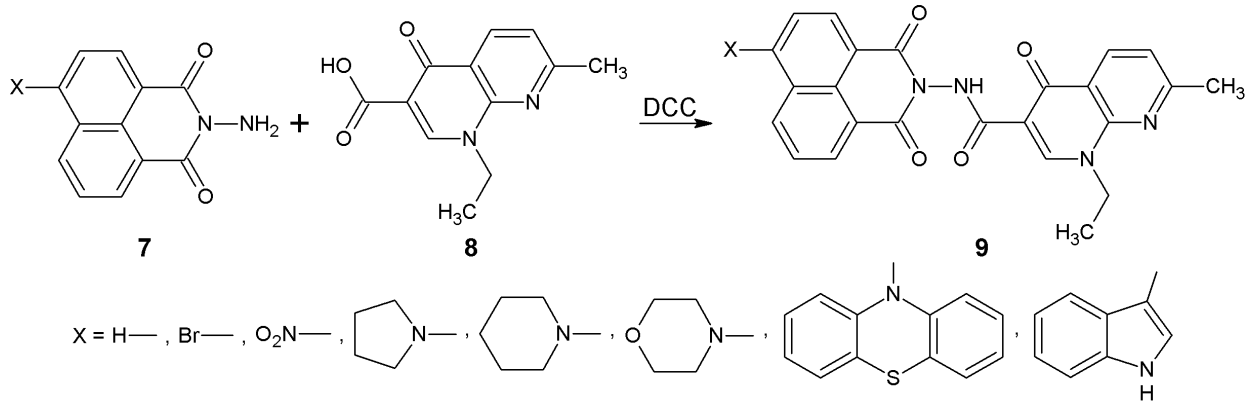
Scheme 2. Synthesis of 6-nitro-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione.



Scheme 3. Synthesis of 6-substituted 1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-diones.



Scheme 4. Synthesis of 6-substituted 2-amino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-diones.



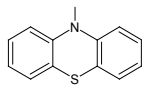
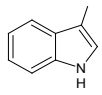
Scheme 5. Synthesis of 6-substituted *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamides.

its 6-substituted derivatives, that of compounds 6 will be used.

The corresponding 1,8-naphthalic anhydride (6) is treated with hydrazine hydrate (Scheme 4). This leads to the formation of different 1,8-naphthalimide derivatives (7).

The planned 1,8-naphthalimide derivatives with nalidixic acid (9) are synthesized according to Scheme 5. The reaction is performed very smoothly at a room temperature and ambient pressure. The reaction proceeds with the highest yield using tetrahydrofuran solvent. Ethyl acetate, dioxane, acrylonitrile and dimethyl

Table 1. Physicochemical parameters of compounds 7a - 7h.

№ [a]	X	IUPAC systematic name	Yield, %	M. p., °C	R_f [b]
7a	H-	2-amino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione	96	211-212	0.67
7b	Br-	2-amino-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione	87	167-168	0.54
7c	O_2N-	2-amino-6-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione	87	261-262	0.43
7d		2-amino-6-(pyrrolidin-1-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione	92	235-236	0.55
7e		2-amino-6-(piperidin-1-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione	85	158-159	0.51
7f		2-amino-6-(morpholin-4-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione	74	214-215	0.61
7g		2-amino-6-(10*H*-phenothiazin-10-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione	95	132-133	0.49
7h		2-amino-6-(1*H*-indol-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione	80	161-162	0.45

a The compounds numbering is in accordance with Scheme 4;
b Eluent system (vol. ratio): ethyl acetate : petroleum ether = 1:2

formamide are also used but the yields with these solvents are lower.

The physicochemical parameters of the synthesized 1,8-naphthalimides (7a - 7h) and their derivatives (9a - 9h) with nalidixic acid (8) are shown in Table 1 and Table 2. Compounds 7a - 7h and 9a - 9h are structurally characterized by an elemental analysis, IR, ^{1}H- and ^{13}C-NMR spectroscopy. The results obtained are presented in Tables 3 - 6, respectively.

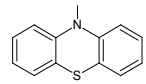
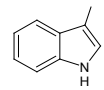
The antimicrobial activity of the synthesized 6-substituted *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamides (9a-9h) is determined against Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633, Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NTCC 6017, the yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763 and the molds *Penicillium chrysogenum* and *Aspergillus niger*. The data obtained from these analyses is presented in Table 7.

Compounds 9a and 9h show an antimicrobial activity towards both Gram-positive and Gram-negative bacteria tested. The 9a product is most effective against *Escherichia coli* (27.5 mm), *Salmonella abony* (23.9 mm) and *Pseudomonas aeruginosa* (20.6 mm). Compound 9h is most active towards *Bacillus subtilis* (18.6mm). Both substances possess antifungal activity against *Candida albicans* (17.5 mm and 10.4 mm, respectively) and *Saccharomyces cerevisiae* (13.1 mm and 12.8 mm, respectively). Compound 9a exhibits a weak activity against the mold *Penicillium chrysogenum*.

Compounds 9b, 9c, 9d and 9g demonstrate a weak activity against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. With regard to the Gram-negative bacteria used, it is found that these compounds are active against *Pseudomonas aeruginosa* (9b - 15.6 mm, 9c - 18.2 mm, 9d - 13.1 mm and 9g - 20.3 mm), but they have no effect against *Escherichia coli* and *Salmonella abony*. Compounds 9c and 9g are weakly active against *Candida albicans* (12.1 mm and 10.3 mm, respectively) and *Saccharomyces cerevisiae* (15 mm and 10.4 mm, respectively). No activity is found against the molds *Penicillium chrysogenum*, *Aspergillus niger*.

Product 9e shows a good activity against *Bacillus subtilis* (18.8 mm), *Escherichia coli* (18.2 mm) and

Table 2. Physicochemical parameters of compounds 9a - 9h.

№ [a]	X	IUPAC systematic name	Yield, %	M. p., °C	Rf [b]
9a	H-	*N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide	49	205-206	0.53
9b	Br-	*N*-(6-bromo-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide	69	135-136	0.48
9c	O_2N-	*N*-(6-nitro-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide	82	200-201	0.37
9d		*N*-(6-pyrrolidin-1-yl -1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide	45	187-188	0.51
9e		*N*-(6-piperidin-1-yl -1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide	91	142-143	0.46
9f		*N*-(6-morpholin-4-yl -1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide	47	192-193	0.53
9g		*N*-(6-10*H*-phenothiazin-10-yl -1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide	83	121-122	0.40
9h		*N*-(6-1*H*-indol-3-yl -1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide	55	141-142	0.39

a The compounds numbering is in accordance with Scheme 5;

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

Table 3. Elemental analysis data of compounds 7a - 7h.

№	Molecular formula / FW	Elemental analysis, %									
		Calculated					Found				
		C	H	N	S	Br	C	H	N	S	Br
7a	$C_{12}H_8N_2O_2$ 212.20	67.92	3.80	13.20			67.78	3.65	13.06		
7b	$C_{12}H_7BrN_2O_2$ 291.10	49.51	2.42	9.62		27.45	49.33	2.18	9.54		27.19
7c	$C_{12}H_7N_3O_4$ 257.20	56.04	2.74	16.34			55.88	2.47	16.08		
7d	$C_{16}H_{15}N_3O_2$ 281.31	68.31	5.37	14.94			68.19	5.31	14.66		
7e	$C_{17}H_{17}N_3O_2$ 295.34	69.14	5.80	14.23			68.87	5.63	13.97		
7f	$C_{16}H_{15}N_3O_3$ 297.31	64.64	5.09	14.13			64.55	4.94	13.88		
7g	$C_{24}H_{15}N_3O_2S$ 409.46	70.40	3.69	10.26	7.83		70.19	3.53	10.06	7.58	
7h	$C_{20}H_{13}N_3O_2$ 327.34	73.38	4.00	12.48			73.24	3.90	12.33		

Table 4. Elemental analysis data of compounds 9a - 9h.

№	Molecular formula / FW	Elemental analysis, %									
		Calculated					Found				
		C	H	N	S	Br	C	H	N	S	Br
9a	$C_{24}H_{18}N_4O_4$ 426.42	67.60	4.25	13.14			67.57	4.15	13.01		
9b	$C_{24}H_{17}BrN_4O_4$ 505.32	57.04	3.39	11.09		15.81	56.89	3.21	11.00		15.58
9c	$C_{24}H_{17}N_5O_6$ 471.42	61.15	3.63	14.86			61.01	3.42	14.73		
9d	$C_{28}H_{25}N_5O_4$ 495.53	67.87	5.09	14.13			67.67	4.95	13.88		
9e	$C_{29}H_{27}N_5O_4$ 509.56	68.36	5.34	13.74			68.07	5.23	13.61		
9f	$C_{28}H_{25}N_5O_5$ 511.53	65.74	4.93	13.69			65.64	4.74	13.55		
9g	$C_{36}H_{25}N_5O_4S$ 623.68	69.33	4.04	11.23	5.14		69.22	3.96	11.01	4.99	
9h	$C_{32}H_{23}N_5O_4$ 541.56	70.97	4.28	12.93			70.81	4.04	12.76		

Table 5. Spectral data of compounds 7a - 7h.

№	IR (KBr, cm^{-1})	^{1}H-NMR (δ, $CDCl_3$, ppm)	^{13}C-NMR [a] (δ, $CDCl_3$, ppm)
7a	3365, 3225, 3061, 1705, 1583, 1370	5.45 (s, NH_2), 7.68-8.57 (m, 6H)	120.8, 126.8, 127.1, 131.6, 131.7, 134.5, 170.1
7b	3348, 3220, 3065, 1714, 1665, 1580, 1367	5.56 (s, NH_2), 7.69-8.62 (m, 5H)	117.7, 122.9, 123.4, 126.7, 127.1, 129.4, 130.8, 131.4, 131.9, 132.8, 147.07, 161.5, 161.7
7c	3353, 3331, 3068, 1705, 1669, 1583, 1531, 1350	5.61 (s, NH_2), 7.65-8.53 (m, 5H)	115.6, 120.3, 122.4, 127.2, 128.1, 130.3, 131.6, 132.1, 133.3, 134.1, 148.1, 165.5, 165.8
7d	3359, 3335, 3065, 1712, 1674, 1581, 1375	5.48 (s, NH_2), 7.66-8.51 (m, 5H)	23.45, 26.6, 116.6, 119.4, 121.9, 127.1, 128.3, 130.4, 132.1, 132.3, 135.3, 149.5, 168.1, 168.3
7e	3362, 3228, 3062, 1716, 1696, 1580, 1371	5.46 (s, NH_2), 7.65-8.49 (m, 5H)	19.96, 23.5, 24.9, 116.3, 120.7, 121.6, 127.5, 130.3, 131.5, 133.4, 134.1, 135.6, 150.6, 167.5, 168.1
7f	3360, 3226, 3065, 1713, 1693, 1579, 1370	5.45 (s, NH_2), 7.66-8.54 (m, 5H)	22.46, 25.8, 117.3, 120.4, 122.2, 126.5, 130.3, 130.9, 133.2, 135.5, 136.6, 150.6, 167.7, 168.6
7g	3363, 3333, 3061, 1704, 1681, 1581, 1372	5.52 (s, NH_2), 7.70-8.62 (m, 5H)	111.9, 116.7, 117.3 120.4, 122.3, 122.8, 131.6, 131.8, 132.1, 133.4, 135.6, 141.2, 148.2, 150.8, 170.2, 171.3
7h	3359, 3232, 3063, 1700, 1679, 1580, 1370	5.53 (s, NH_2), 7.69-8.58 (m, 5H)	112.3, 114.6, 115.5, 117.4, 119.6, 123.9, 130.2, 130.4, 133.4, 137.5, 140.3, 149.5, 150.3, 169.4, 170.5

a These assignments are confirmed by the DEPT-135 spectral data.

Table 6. Spectral data of compounds 9a - 9h.

№	IR (KBr, cm^{-1})	^{1}H-NMR (δ, $CDCl_3$, ppm)	^{13}C-NMR [a] (δ, $CDCl_3$, ppm)
9a	3321. 3043, 2984, 1727, 1680, 1651, 1586-1371	1.12 (s, 3H, CH_3), 2.17 (t, 2H, CH_2), 7.56-8.61 (m, 9H), 9.15 (s, NH)	18.5, 20.1, 32.3, 121.6, 127.2, 131.8, 135.3, 154.4, 168.7, 172.2
9b	3343, 3032, 2981, 1718, 1681, 1648, 1585-1370	1.16 (s, 3H, CH_3), 2.15 (t, 2H, CH_2), 7.61-8.65 (m, 9H), 8.71 (s, NH)	18.1, 19.3, 27.6, 120.5, 126.3, 129.4, 133.6, 154.1, 167.6, 171.3
9c	3276, 3033, 2979, 1716, 1680, 1649, 1585, 1530, 1371, 1350	1.21 (s, 3H, CH_3), 2.15 (t, 2H, CH_2), 7.56-8.47 (m, 9H), 8.50 (s, NH)	18.8, 20.3, 31.4, 122.5, 127.4, 130.9, 134.7, 154.5, 168.9, 173.1
9d	3337, 3035, 2980, 1725, 1681, 1652, 1587-1370	1.35 (s, 3H, CH_3), 2.32 (t, 2H, CH_2), 7.68-8.57 (m, 9H), 8.88 (s, NH)	14.5, 17.3, 18.9, 21.1, 32.3, 123.4, 128.5, 131.6, 135.6, 154.8, 169.1, 174.2
9e	3345, 3029, 2983, 1728, 1682, 1650, 1586, 1371	1.32 (s, 3H, CH_3), 2.26 (t, 2H, CH_2), 7.63-8.66 (m, 9H), 8.85 (s, NH)	12.7, 14.8, 17.4, 18.8, 21.3, 32.6, 123.7, 129.3, 134.5, 136.2, 155.3, 170.2, 175.5
9f	3344, 3031, 2981, 1731, 1681, 1650, 1585, 1371	1.36 (s, 3H, CH_3), 2.35 (t, 2H, CH_2), 7.60-8.52 (m, 9H), 8.63 (s, NH)	15.3, 17.6, 18.9, 22.1, 31.6, 124.3, 130.2, 135.3, 138.1, 155.5, 170.3, 175.6
9g	3299, 3035, 2979, 1721, 1679, 1651, 1582, 1370	1.11 (s, 3H, CH_3), 2.14 (t, 2H, CH_2), 7.51-8.67 (m, 9H), 8.12 (s, NH)	17.9, 19.6, 32.4, 120.8, 127.4, 130.3, 134.4, 155.2, 168.7, 174.6
9h	3338, 3039, 2985, 1726, 1680, 1651, 1583, 1371	1.20 (s, 3H, CH_3), 2.22 (t, 2H, CH_2), 7.54-8.65 (m, 9H), 8.54 (s, NH)	18.0 19.8, 33.6, 121.2, 128.1 130.5, 134.6, 153.7, 169.2, 175.3

a These assignments are confirmed by the DEPT-135 spectral data.

Table 7. Antimicrobial activity of compounds 9a - 9h.

Microorganism	Inhibition zone (mm)							
	9a	9b	9c	9d	9e	9f	9g	9h
Staphylococcus aureus ATCC 6538	17.5	11.3	15.0	9.9	-	-	11.5	16.8
Bacillus subtilis ATCC 6633	19.8	13.1	10.4	10.4	18.8	-	14.0	18.6
Escherichia coli ATCC 8739	27.5	-	-	-	18.2	-	-	17.3
Pseudomonas aeruginosa ATCC 9027	20.6	15.6	18.2	13.1	11.8	-	20.3	17.3
Salmonella abony NTCC 6017	23.9	-	-	-	15.5	-	-	16.5
Candida albicans ATCC 10231	17.5	-	12.1	-	-	-	10.3	10.4
Saccharomyces cerevisiae ATCC 9763	13.1	-	15.8	-	-	-	10.4	12.8
Penicillium chrysogenum	11.4	-	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	-	-	-	-

- No zone of inhibition.

Salmonella abony (15.5 mm).

Compound 9f exhibits no activity in respect to the test microorganisms used.

CONCLUSIONS

The synthesis of new carboxamides (9a-9h) based on the reaction of the 6-substituted 2-amino-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-diones (7a-7h) with nalidixic acid (8) is presented herein. The structures of the synthesized compounds are proven through their physicochemical parameters as well as by IR, ^{1}H and ^{13}C NMR spectral data. The antimicrobial study of the products obtained shows that the most effective compounds refer to *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (9a) and *N*-(6-1*H*-indol-3-yl-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (9h), which exhibit an antimicrobial activity against both Gram-positive and Gram-negative bacteria tested. The compound *N*-(6-piperidin-1-yl-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (9e) is characterized by a good activity towards *Bacillus subtilis*, *Escherichia coli* and *Salmonella abony*.

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