Microwave-Assisted Synthesis of 1,3′-Diaza-flavanone/flavone and Their Alkyl Derivatives with Antimicrobial Activity

Nuran Kahriman,[a] Nagihan Yilmaz Iskender,[a] Murat Yücel,[a] Nurettin Yayli,[a]* Emine Demir,[b] and Zihni Demirbağ[b]

[a]Department of Chemistry, Faculty of Science, Karadeniz Technical University, 61080, Trabzon, Turkey
[b]Department of Biology, Faculty of Science, Karadeniz Technical University, 61080, Trabzon, Turkey
*E-mail: yayli@ktu.edu.tr

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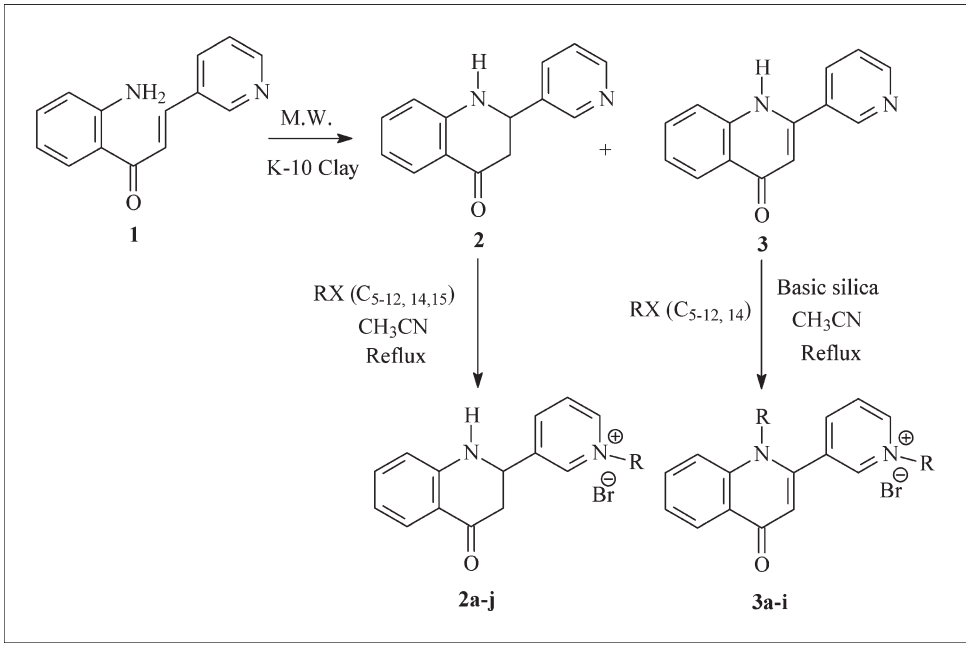

A simple environmentally friendly solid-phase microwave-assisted method was used to synthesis of the 1,3′-diazaflavanone (**2**) and 1,3′-diazaflavone (**3**) from the cyclization of 2′-amino (*E*)-3″-azachalcone (**1**). Ten new *N*-alkyl ($C_{5–12,14,15}$)-substituted 1,3′-diazaflavanonium bromides (**2a–j**) were prepared from compound **2** with corresponding alkyl halides in acetonitrile under reflux. In addition, nine new *N*,*N*′-dialkyl ($C_{5–12,14}$)-substituted 1,3′-diazaflavonium bromides (**3a–i**) were also synthesized from compound **3** with corresponding alkyl halides using basic silica in acetonitrile. The antimicrobial activities of compounds **1–3**, **2a–j**, and **3a–i** were tested against Gram-positive (G+) (*Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus, and Enterococcus faecalis*) and Gram-negative (G−) (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhimirium*, *Yersinia pseudotuberculosis*, and *Enterobacter cloaceae*) microorganisms. They showed good antimicrobial activity against the Gram-positive bacteria tested with the minimal inhibitory concentration values less than 7.8 μg/mL in most cases. The optimum length of the alkyl chain for better and broader activity is situated in the range of 9–12 carbon atoms in the series of compounds **2a–j** and five to six carbon atoms in the series of compounds **3a–i**. The nonalkylated compounds **1–3** were not effective, as were the ones alkylated with five or six C alkyl groups (**2a** and **2b**) and 8–13 C alkyl groups for *N*,*N*′-dialkyl compounds (**3c–3i**). The antimicrobial activity increased as the length of the alkyl substitution increased from 8 to 12 carbons in compounds **2a–j**. However, antimicrobial activity decreased as the length of the alkyl substitution increased from 7 to 13 carbons in compounds **3c–i**.

INTRODUCTION

Flavanones and flavones are a group of natural compounds widely present in the plant kingdom and show various pharmacological activities [1]. 1,3′-Diazaflavanone [2-(pyridin-3-yl)-1,2,3,4-tetrahydroquinolin-4(1H)-one] and 1,3′-diazaflavone [2-pyridin-3-ylquinolin-4(1H)-one] are analogous of flavanones and flavones [1–

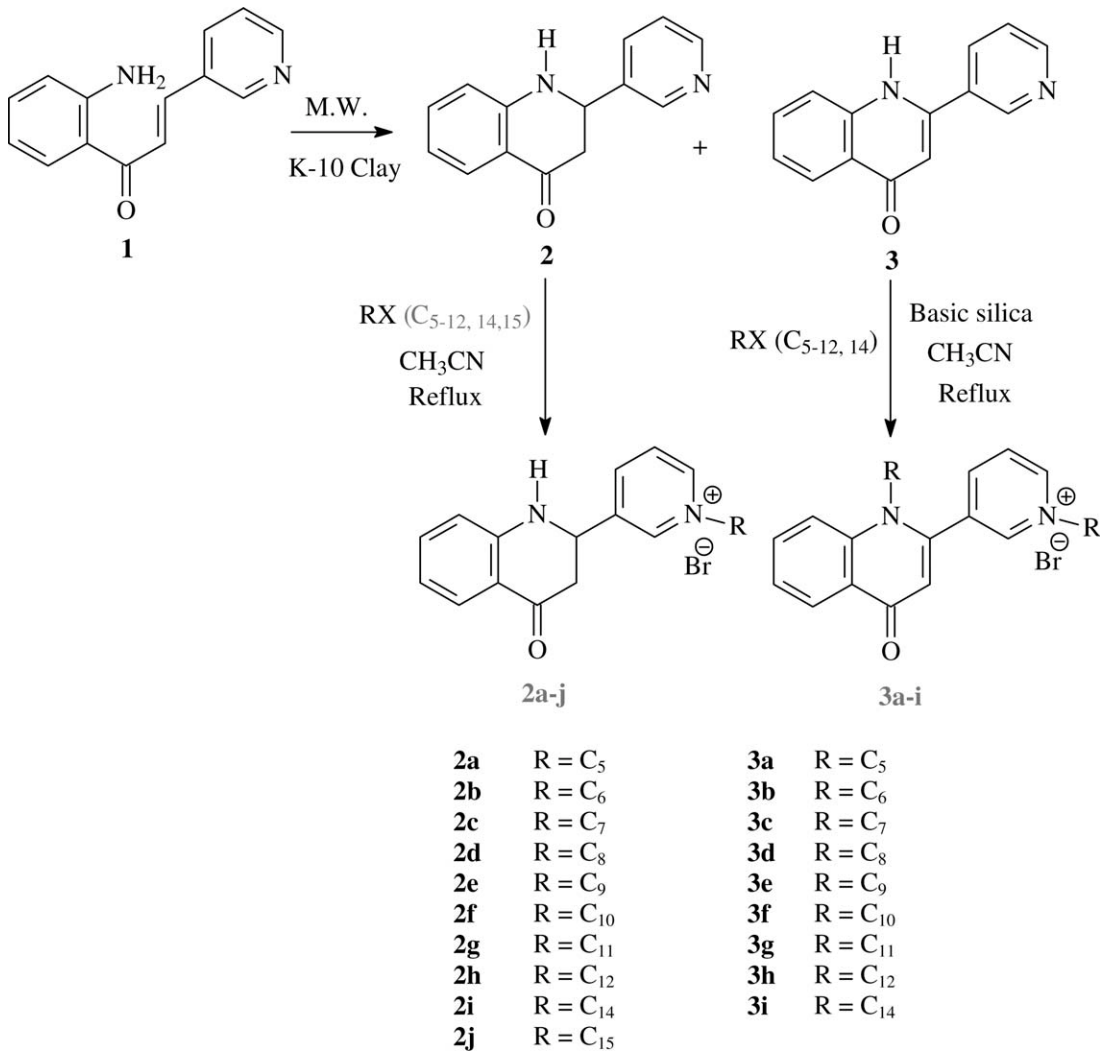

Figure 1.

5] with annular nitrogen atoms. The quinolin-4(1H)-one ring is found in many organic compounds such as dyes and pharmaceuticals [4–6]. Azaflavanones or azaflavones can be prepared by base- or acid-catalyzed reactions. Many of the synthetic procedures for the synthesis of flavanones or azaflavanones/azaflavones involve the use of corrosive reagents such as strong alkali or orthophosphoric acid [6–11]. Furthermore, many of them are of limited synthetic scope because of lower yields and longer reaction time. Synthesis of 1,3′-diazaflavanone and 1,3′-diazaflavone have been mentioned through the reduction by CO of the corresponding 2-nitroazachalcone catalyzed by $Ru_3(CO)_{12}$-DIAN-Me in EtOH-H_2O [12].

In the recent years, microwave irradiation has become a very powerful tool in organic synthesis [12–14] and been used by many organic chemists. Microwave-assisted reactions take place selectively in solvent- and solid-phase condition in a very short time, with cleaner reaction, greater selectivity, and improved yields [12,13].

In continuation of our studies on the synthesis of heterocyclic compounds with medicinal potential from 2′-amino (*E*)-3″-azachalcone, we report an efficient and simple method for the synthesis of 1,3′-diazaflavanones and 1,3′-diazaflavones through the cyclization of the corresponding azachalcone using K-10 clay under solid-phase conditions (Fig. 1). In our previous work, due to a biological interest, 1,4′-diazaflavone and their *N*-alkyl derivatives were reported and showed good antimicrobial activity against Gram-positive bacteria [15]. One simple method has been mentioned for the synthesis of 2-phenyl-2,3-dihydroquinolin-4(1H)-one from 2′-amino (*E*)-chalcone by microwave using silica-supported sodium hydrogen sulphate and various catalysts under solventless conditions [11]. However, the synthesis of 1,3′-diazaflavanone and 1,3′-diazaflavones has not been

Table 1

Physicochemical data of compounds **2–2a–j** and **3–3a–i**.

Comp.	IR ($CHCl_3$, cm^{-1}) NH	IR ($CHCl_3$, cm^{-1}) C=O	Yield (%)	m.p. (°C)	UV–vis λ_{nm} (log ε)			TLC[a] (R_f)
2	3466	1668	55	155–158	376 (3.5)	256 (3.9)	234 (4.2)	0.52
2a	3407	1668	91	Oily	360 (3.5)	256 (4.1)	230 (4.4)	0.72
2b	3406	1671	92	Oily	358 (3.4)	258 (3.9)	230 (4.3)	0.69
2c	3407	1671	82	Oily	352 (3.6)	258 (4.2)	230 (4.5)	0.69
2d	3412	1669	90	Oily	354 (3.5)	258 (4.0)	230 (4.3)	0.68
2e	3407	1669	92	Oily	352 (3.4)	258 (4.0)	230 (4.3)	0.69
2f	3412	1669	89	122–125	348 (3.5)	256 (4.1)	230 (4.4)	0.72
2g	3423	1668	87	Oily	362 (3.4)	258 (3.9)	230 (4.2)	0.83
2h	3412	1668	89	119–122	364 (3.5)	260 (4.0)	230 (4.3)	0.81
2i	3418	1672	73	125–128	364 (3.5)	260 (4.0)	230 (4.3)	0.75
2j	3412	1669	72	168–171	366 (3.6)	260 (4.1)	230 (4.5)	0.74
3	3401	1601	60	176–179	332 (3.8)	250 (4.2)	–	0.74
3a	–	1587	83	Oily	304 (4.3)	266 (4.3)	246 (4.3)	0.45
3b	–	1587	77	Oily	302 (4.0)	266 (4.3)	244 (4.3)	0.50
3c	–	1587	71	71–74	304 (4.5)	266 (4.8)	244 (4.8)	0.53
3d	–	1587	88	Oily	304 (4.4)	290 (4.4)	246 (4.6)	0.55
3e	–	1587	80	Oily	304 (4.3)	290 (4.3)	244 (4.6)	0.72
3f	–	1587	75	65–68	302 (4.1)	292 (4.1)	244 (4.3)	0.72
3g	–	1587	79	68–71	304 (4.0)	292 (4.0)	244 (4.3)	0.72
3h	–	1587	90	107–109	304 (4.1)	292 (4.1)	244 (4.4)	0.75
3i	–	1587	85	57–60	304 (4.0)	294 (4.0)	244 (4.3)	0.75

[a] Ethyl acetate-methanol (2:0.5) for compounds **2–2a–j** and ethyl acetate-methanol (3:0.5) for compounds **3–3a–i**.

mentioned starting from 2′-amino (*E*)-3″-azachalcone by microwave-assisted methods.

N-Alkyl derivatives of azachalcones and diazaflavones have been reported to have a wide variety of biological activity such as, antibacterial, antimicrobial, antituberculostatic, and anti-inflammatory [5,16–21]. In view of our continuous interest on antimicrobial agents, we synthesized a series of *N*-alkyl ($C_{5–12,14,15}$) and *N*,*N*′-dialkyl ($C_{5–12,14}$) substituted-1,3′-diaza-flavanonium/flavonium bromides (**2a–j** and **3a–i**) in this respect. We wanted to determine the influence of the length of the carbon chain in the *N*-alkyl substituent.

This work deals with the synthesis, spectral characterization, and results of antimicrobial activity assays of 2′-amino (*E*)-3″-azachalcone (**1**), 1,3′-diazaflavanone (**2**), 1,3′-diazaflavone (**3**), *N*-alkyl ($C_{5–12,14,15}$) substituted-1,3′-diazaflavanonium (**2a–j**), and *N*,*N*′-dialkyl ($C_{5–12,14}$) substituted-1,3′-diazaflavonium bromides (**2a–j**).

RESULTS AND DISCUSSION

The scheme shows the synthetic approach chosen for the preparation of 1,3′-diazaflavanone (**2**) and 1,3′-diazaflavone (**3**) through the cyclization [13–21] of corresponding 2′-amino (*E*)-3″-azachalcone (**1**) using K-10 clay under solventless conditions by using microwave at low temperature and power (85°C, 350 W), which furnished the 1,3′-diazaflavanone (**2**) as the major product (55% yield) beside 1,3′-diazaflavone (**3**). When we did the reaction at high temperature (500 W for 20 min at 110°C), 1,3′-diazaflavone (**3**) was the main product of the reaction (60% yield).

N-Alkyl derivatives of azachalcones, diazaflavanones, and diazaflavones attract widespread interest because many of them have exhibited a wide variety of biological activities [10,11,14,16–21]. *N*-Alkyl and *N*,*N*′-dialkyl derivatives of 1,3′-diazaflavanone and 1,3′diazaflavones were prepared from the corresponding diazaflavanone with *n*-bromoalkanes (1-bromopentane, 1-bromohexane, 1-bromoheptane, 1-bromooctane, 1-bromononane, 1-bromodecane, 1-bromoundecane, 1-bromododecane, 1-bromotetradecane, and 1-bromopentadecane) in acetonitrile solution by reflux [10,11,14,16–21]. All the synthesized compounds (**2a–j**, **3a–i**) were characterized on the basis of spectral data studies (^1H, ^{13}C, APT, ^1H-^1H COSY NMR, ACD-NMR, FTIR, UV, LC-MS/MS, and Elemental Analysis), whose results were in agreement with the proposed structure (Tables 1–5). Based on the above observations, the complete chemical shift assignments for **2a–j** and **3a–i** were deduced to be 1-alkyl($C_{5–12,14–15}$)-3-(4-oxo-1,2,3,4-tetrahydroquinolin-2-yl)pyridinium bromide and 1-alkyl($C_{5–12,14}$)-3-(1-alkyl($C_{5–12,14}$)-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide, respectively.

Antimicrobial activity. The antimicrobial activities of compounds **1–3**, **2a–j**, **3a–i**, kanamycine, and gentamycine were assayed against the Gram-positive (G+) and Gram-negative (G-) micro-organisms. Antimicrobial

Table 2

LC MS/MS and elemental analyses data of compounds **2–2a–j** and **3–3a–i**.

		LC MS/MS (%)				Elemental Analyses Data (%)					
						C		H		N	
Comp.	Formula	$[M(^{79}Br)]^+$	$[M(^{81}Br)]^+$	$[M-^{79}Br]^+$	$[M-^{81}Br+1]^+$	Calculated	Found	Calculated	Found	Calculated	Found
2	$C_{14}H_{12}N_2O$	224 (04)[a]	225 (100)[b]	–	–	74.98	74.976	5.39	5.336	12.49	12.600
2a	$C_{19}H_{23}N_2OBr$	374 (05)	376 (05)	295 (100)	296 (48)	60.81	60.846	6.18	6.100	7.46	7.349
2b	$C_{20}H_{25}N_2OBr$	388 (05)	390 (04)	309 (100)	310 (60)	61.70	61.850	6.47	6.579	7.20	7.234
2c	$C_{21}H_{27}N_2OBr$	402 (06)	404 (05)	323 (100)	324 (25)	62.53	62.511	6.75	6.789	6.94	6.892
2d	$C_{22}H_{29}N_2OBr$	416 (05)	418 (03)	337 (40)	338 (12)	63.31	63.305	7.00	6.922	6.71	6.759
2e	$C_{23}H_{31}N_2OBr$	430 (08)	432 (03)	351 (100)	352 (68)	64.03	64.098	7.24	7.310	6.49	6.419
2f	$C_{24}H_{33}N_2OBr$	444 (05)	446 (03)	365 (100)	366 (74)	64.71	64.692	7.47	7.557	6.29	6.272
2g	$C_{25}H_{35}N_2OBr$	458 (10)	460 (05)	379 (100)	380 (75)	65.35	65.405	7.68	7.433	6.10	6.145
2h	$C_{26}H_{37}N_2OBr$	472 (10)	474 (20)	393 (100)	394 (30)	65.95	65.863	7.88	7.915	5.92	5.898
2i	$C_{28}H_{41}N_2OBr$	500 (07)	502 (03)	421 (100)	422 (95)	67.05	67.110	8.24	8.372	5.59	5.501
2j	$C_{29}H_{43}N_2OBr$	514 (05)	516 (03)	435 (100)	436 (38)	67.56	67.601	8.41	8.460	5.43	5.477
3	$C_{14}H_{10}N_2O$	222 (22)[a]	225 (100)[b]	–	–	75.66	75.473	4.54	4.541	12.60	12.710
3a	$C_{24}H_{31}N_2OBr$	442 (08)	444 (05)	363 (100)	364 (88)	65.01	65.134	7.05	7.074	6.32	6.352
3b	$C_{26}H_{35}N_2OBr$	470 (05)	472 (04)	391 (100)	392 (90)	66.24	66.185	7.48	7.531	5.94	5.963
3c	$C_{28}H_{39}N_2OBr$	498 (06)	500 (04)	419 (100)	420 (98)	67.32	67.167	7.87	8.079	5.61	5.647
3d	$C_{30}H_{43}N_2OBr$	526 (05)	528 (03)	447 (100)	448 (98)	68.30	68.408	8.22	8.259	5.31	5.285
3e	$C_{32}H_{47}N_2OBr$	554 (05)	556 (04)	475 (100)	476 (60)	69.17	69.344	8.53	8.583	5.04	5.120
3f	$C_{34}H_{51}N_2OBr$	582 (04)	584 (03)	503 (100)	504 (80)	69.96	69.825	8.81	8.906	4.80	4.747
3g	$C_{36}H_{55}N_2OBr$	610 (04)	612 (03)	531 (100)	532 (68)	70.68	70.466	9.06	9.187	4.58	4.561
3h	$C_{38}H_{59}N_2OBr$	638 (04)	640 (03)	559 (100)	560 (60)	71.34	71.316	9.29	9.303	4.38	4.333
3i	$C_{42}H_{67}N_2OBr$	695 (05)	697 (03)	616 (100)	617 (50)	72.49	72.568	9.70	9.724	4.03	4.006

[a] $[M]^+$
[b] $[M+1]^+$

activities of studied bacteria were assessed qualitatively and quantitatively by evaluating the presence of inhibition zones and minimal inhibitory concentration (MIC) values [22–25]. All the compounds except compounds **1–2**, **2a–b** showed antimicrobial activity against G+ bacteria. However, none of the compounds showed antimicrobial activity against the G- bacteria tested. Compounds **1–3**, **2a–b**, and **3d–i** were inactive against all test microorganisms. The results are given in Table 5. The maximal inhibition zones and MIC values for bacterial strains, which were sensitive to the compounds **2d–h** and **3a–b**, were in the range of 6–14 mm, and from 7.8 to 200 μg/mL, respectively (Table 5). It is clear that the length of the alkyl chain influences the broadening of the spectrum of antimicrobial activity and MIC of value of the investigated compounds. The optimum length of the alkyl chain for better and broader activity is situated in the range of 8–12 carbon atoms in the series of compounds **2a–j** and five to six carbon atoms in the series of compounds **3a–i**.

EXPERIMENTAL

General. NMR spectra were recorded on a Varian Mercury NMR at 200 MHz in $CDCl_3$-CD_3OD (10:1). NMR data assignments were based on ^1H, ^{13}C, APT, ^1H-^1H COSY, and ACD NMR program. The mass-spectral analyses were carried out on a Micromass Quattro LC-MS/MS spectrophotometer with 3.65 kV capillary voltage, 52 V cone, 1 extractor value, 0.0 lens value, 80°C source temperature, and 120°C desolvation temperature. The elemental analyses were performed on a Costech ECS 4010 instrument. Infrared spectra were obtained with a Perkin-Elmer 1600 FTIR (4000–400 cm^{-1}) spectrometer. Melting points were determined by using a Thermo-var apparatus fitted with a microscope and uncorrected. UV–vis spectral analyses were carried out on a Unicam UV2–100 at 25°C. The reactions were carried out in Milestone microwave instrument (350 and 500 W). Thin-layer chromatography (TLC) was carried out on Merck precoated 60 Kieselgel F_{254} analytical aluminum acidic or basic plates.

MATERIALS AND METHODS

2-Aminoacetophenone, 3-pyridine carbaldehyde, and bromoalkanes ($C_{5–12,14,15}$) were purchased from Aldrich/Fluka and used without further purification. The solvents (chloroform, *n*-hexane, ethanol, methanol, acetonitrile, ethyl acetate, and diethyl ether) used were either of analytical grade or bulk solvents distilled before use. The known compound **1** [26] was prepared according to the literature [27] ($R_f = 0.6$, ethyl acetate).

General procedure for synthesis of compounds 2 and 3. 2′-Amino (*E*)-3″-azachalcone (**1**) (0.005 mol each) was dissolved in chloroform and was uniformly adsorbed on the surface of K-10 clay [3] (15 g) in a pyrex round-bottomed flask. The solvent was evaporated under vacuum, and then the adsorbed material was transferred to a pyrex tube (2-cm diameter, 30 mL) and inserted inside the Milestone microwave

Table 3

^{1}H NMR data of compounds **2–2a–j** and **3–3a–i**.

	^{1}H NMR, δ ppm ($CDCl_3$-CD_3OD, 10:1), $J^{b,c}$ (Hz)													
Comp.	NH	H_2	H_3	H_5	H_6	H_7	H_8	$H_{2'}$	$H_{4'}$	$H_{5'}$	$H_{6'}$	$N_{Pry.}$-(CH_2)-N_1-(CH_2)-	-$(CH_2)_n$- n: 3–10, 12–13	─(CH_3)
2	4.7, bs	4.8	2.8	7.9	6.8	7.3	6.8	8.7, bs	8.6	7.8	7.4	–	–	–
2a	7.3, bs	5.1	2.9	7.8	7.3	6.7	7.1	9.5, bs	9.0	8.0	8.6	4.7	1.4–1.9, 6H	0.8
2b	7.4, bs	5.1	2.9	7.7	7.2	6.7	7.2	9.5, bs	9.0	8.0	8.6	4.7	1.2–1.9, 8H	0.8
2c	7.3, bs	5.0	2.8	7.5	7.2	6.8	7.0	9.5, bs	9.0	7.9	8.5	4.6	1.1–1.9, 10H	0.7
2d	7.3, bs	5.0	2.8	7.6	7.2	6.6	7.1	9.5, bs	9.0	7.9	8.5	4.6	1.1–1.9, 12H	0.7
2e	7.2, bs	5.0	2.8	7.5	7.2	6.7	7.0	9.4, bs	8.9	7.9	8.5	4.6	1.1–1.9, 14H	0.7
2f	7.2, bs	5.0	2.8	7.5	7.1	6.6	7.0	9.4, bs	8.9	7.9	8.5	4.6	1.1–1.9, 16H	0.7
2g	7.6, bs	5.1	2.9	7.7	7.3	6.7	7.2	9.5, bs,	9.0	8.0	8.6	4.7	1.2–1.9, 18H	0.9
2h	7.4, bs	5.1	2.9	7.6	7.2	6.7	7.1	9.5, bs	9.0	7.9	8.5	4.7	1.2–1.9, 20H	0.8
2i	7.4, bs	5.0	2.9	7.6	7.2	6.6	7.1	9.5, bs	9.0	7.9	8.5	4.7	1.2–1.9, 24H	0.8
2j	7.5, bs	5.1	2.9	7.7	7.3	6.7	7.2	9.6, bs	9.0	8.0	8.6	4.7	1.3–1.9, 26H	0.9
3	12.2, bs	–	6.6, s	8.3	7.4	7.7	7.7	9.0, bs	8.7	7.6	8.2	–	–	–
3a	–	–	8.0, s	8.2	7.6	7.7	7.9	10.5, bs	9.4	8.1	9.2	4.6/5.1	1.4–2.0, 12H	1.0/0.9
3b	–	–	8.0, s	8.2	7.6	7.7	7.9	10.6, bs	9.4	8.1	9.2	4.5/5.1	1.3–2.1, 16H	0.8/0.9
3c	–	–	8.0, s	8.2	7.6	7.7	7.9	10.4, bs	9.4	8.1	9.3	4.6/5.2	1.2–2.1, 20H	0.8/0.9
3d	–	–	8.0, s	8.2	7.6	7.7	7.9	10.5, bs	9.4	8.1	9.2	4.6/5.2	1.2–2.1, 24H	0.8/0.9
3e	–	–	8.0, s	8.2	7.5	7.7	7.9	10.4, bs	9.4	8.1	9.3	4.5/5.1	1.2–2.0, 28H	0.8/0.9
3f	–	–	8.0, s	8.2	7.5	7.7	7.9	10.4, bs	9.4	8.1	9.3	4.5/5.3	1.2–2.1, 32H	0.8/0.9
3g	–	–	8.0, s	8.2	7.5	7.7	7.9	10.4, bs	9.4	8.1	9.3	4.5/5.2	1.2–2.1, 36H	0.8/0.9
3h	–	–	8.0, s	8.2	7.5	7.7	7.9	10.4, bs	9.4	8.1	9.3	4.6/5.2	1.2–2.1, 40H	0.8/0.9
3i	–	–	8.0, s	8.2	7.5	7.7	7.9	10.4, bs	9.4	8.1	9.2	4.6/5.1	1.2–2.1, 48H	0.8/0.9

[a]Assignment based on ^{1}H, ^{1}H-^{1}H COSY, and comparison with ACD NMR program.

[b]$J_{2–2a–j}$ (Hz): H_2, dd (∼4.6, 5.2); H_3, ABX; H_5, d (∼7.6); H_6, dd (∼7.6, 7.0); H_7, dd (∼7.6, 7.0); H_8; d (∼7.6); $H_{4'}$,d (6.0); $H_{5'}$, dd (∼7.6, 6.0); $H_{6'}$,d (∼7.8); N-(CH_2) -, *t* (∼6.4–7.0); -$(CH_2)_n$ -, m; -(CH_3), t (∼6.4–7.0).

[c]$J_{3–3a–i}$ (Hz): H_5, d (∼7.2–7.8); H_6, dd (∼7.2–7.6, 7.0–8.0); H_7, dd ∼7.0–8.0, 6.8–7.8); H_8, d (∼7.2–8.0); $H_{4'}$, d (∼4.6–8.2); $H_{5'}$, dd (∼7.0–7.6, 6.0–7.8); $H_{6'}$, d (∼5.0–7.8); N-(CH_2) -, t (∼6.2–6.8 and ∼6.8–7.2); -$(CH_2)_n$ -, m; -(CH_3), t (∼6.2–6.6 and ∼6.2–7.2).

Table 4

^{13}C NMR data of compounds 2–2a–j and 3–3a–i.

	^{13}C NMR, δ ppm ($CDCl_3$-CD_3OD, 10:1)[a]																
Comp.	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	$C_{1'}$	$C_{2'}$	$C_{4'}$	$C_{5'}$	$C_{6'}$	$N_{Pry.}$-(CH_2)-N_1-(CH_2)-	-$(CH_2)_n$- n: 3–10, 12–13	-CH_3
2	56.2/ 56.6	46.3/ 46.6	192.7	127.7/ 128.0	119.0/ 119.2	135.7/ 136.0	116.2/ 116.4	151.5	119.3	136.8	148.7/ 148.8	150.1/ 150.3	123.9/ 124.2	134.4/ 134.7	–	–	–
2a	53.8	44.4	191.7	127.0	118.7	136.1	117.2	151.3	118.7	143.4	144.0	144.3	143.2	128.2	62.3	22.2–31.5 (3C)	14.0
2b	53.8	44.4	191.8	127.0	118.8	136.1	117.2	151.3	118.6	143.2	144.0	144.2	143.2	128.3	62.4	22.5–31.7 (4C)	14.2
2c	53.8	44.4	191.7	127.0	118.6	136.0	117.2	151.3	118.7	143.2	144.0	144.0	143.2	128.2	62.4	22.6–31.8 (5C)	14.2
2d	53.5	44.0	191.3	126.7	118.3	135.7	116.8	150.9	118.3	142.9	143.6	144.0	142.8	127.8	62.1	22.3–31.5 (6C)	13.9
2e	53.4	44.0	191.3	126.6	118.2	135.6	116.7	150.9	118.2	142.7	142.9	143.7	142.7	127.9	62.0	22.3–31.9 (7C)	13,9
2f	53.4	44.0	191.3	126.6	118.2	135.6	116.7	150.9	118.2	142.8	143.0	143.7	142.8	127.9	62.0	22.4–31.6 (8C)	13.9
2g	53.4	44.0	191.0	126.7	118.2	135.8	116.9	151.1	118.3	143.0	143.6	144.0	143.0	127.8	62.1	22.5–31.7 (8C)	14.0
2h	53.5	44.0	191.3	126.7	118.3	135.8	116.9	150.9	118.4	143.0	143.6	144.2	142.7	127.8	62.1	25.5–31.8 (8C)	14.0
2i	53.8	44.4	191.7	127.1	118.6	136.1	117.2	151.3	118.7	143.3	144.0	144.4	143.3	128.2	62.4	22.9–32.8 (8C)	14.4
2j	53.6	44.1	191.3	126.8	118.4	135.8	117.0	151.0	118.4	143.1	143.6	144.2	142.7	127.8	62.2	22.6–31.8 (8C)	14.1
3	147.9	107.9	178.9	124.6	124.1	135.4	118.2	140.3	130.3	124.3	147.2	150.3	123.8	132.3	–	–	–
3a	150.3	99.7	163.8	127.7	126.8	130.6	122.3	148.7	121.5	140.5	143.9	143.2	129.1	143.6	62.0/70.5	22.2–31.9 (6C)	13.8/14.1
3b	150.3	99.6	163.7	127.7	126.7	130.6	122.3	148.7	121.4	140.4	143.9	143.1	129.1	143.6	62.0/70.4	22.4–32.1 (8C)	13.9/14.1
3c	150.2	99.3	163.8	127.7	126.8	130.6	122.2	148.7	121.4	140.3	143.8	143.3	129.1	143.6	62.0/70.6	22.5–32.3 (10C)	14.0/14.1
3d	150.1	99.4	163.5	127.8	126.6	130.4	1122.1	148.6	121.2	140.1	144.0	143.1	129.0	143.4	61.8/70.4	22.4–32.1 (10C)	13.9/14.0
3e	150.2	99.5	163.6	127.8	126.7	130.5	1122.2	148.7	121.3	140.2	143.9	143.3	129.0	143.5	61.9/70.5	22.5–31.2 (12C)	14.0/14.1
3f	150.2	99.5	163.6	127.8	126.6	130.4	1122.1	148.6	121.3	140.1	143.9	143.1	129.0	143.5	61.8/70.5	22.5–32.1 (13C)	14.0
3g	150.1	99.4	163.5	127.8	126.6	130.4	1122.1	148.6	121.3	140.1	143.9	143.1	129.0	143.4	61.8/70.4	22.5–32.1 (14C)	14.0
3h	150.1	99.3	163.5	127.8	126.5	130.4	1122.0	148.5	121.2	139.9	144.0	143.1	128.9	143.4	61.8/70.4	22.5–32.1 (12C)	13.9
3i	150.2	99.5	163.7	127.7	126.7	130.6	1122.2	148.7	121.4	140.4	144.1	143.2	129.0	143.6	62.0/70.5	22.6–32.2 (9C)	14.1

[a] Assignment based on APT and comparison with ACD NMR program.

Table 5

Antimicrobial activities of the compounds 1–3, 2a–j and 3a–i against the bacterial strains tested.

Comp.	Stock solution (μg/disk)	*Bs*		*Se*		*Sa*		*Ef*	
		ID	MIC	ID	MIC	ID	MIC	ID	MIC
1	300	–	–	–	–	–	–	–	–
2	300	–	–	–	–	–	–	–	–
2a	300	–	–	–	–	–	–	–	–
2b	300	–	–	–	–	–	–	–	–
2c	300	–	–	8	400	–	–	–	–
2d	300	–	–	13	200	9	400	6	75
2e	300	6	200	13	200	10	75	8	75
2f	300	6	50	15	25	10	50	8	75
2g	300	9	25	14	25	10	25	9	25
2h	300	7	<12.5	8	<12.5	9	<12.5	7	<12.5
2i	300	–	–	7	<12.5	6	<12.5	7	<12.5
2j	300	–	–	–	–	6	200	–	–
3	300	–	–	–	–	–	–	–	–
3a	300	10	<7.8	10	<7.8	11	<7.8	10	<15.6
3b	300	6	<15.6	8	<15.6	8	<15.6	6	<15.6
3c	300	–	–	6	<7.8	–	–	–	–
3d	300	–	–	–	–	–	–	–	–
3e	300	–	–	–	–	–	–	–	–
3f	300	–	–	–	–	–	–	–	–
3g	300	–	–	–	–	–	–	–	–
3h	300	–	–	–	–	–	–	–	–
3i	300	–	–	–	–	–	–	–	–
Ka	300	14	50–25	12	25–12.5	12	100–50	9	200–100
Ge	100	–	–	–	–	–	–	–	–

Gram-positive bacteria (G+) *Bacillus subtilis*: Bs, *Staphylococcus epidermidis*: Se, *Staphylococcus aureus*: Sa, *Enterococcus faecalis*: Ef and Gram-negative bacteria (G−) *Escherichia coli*: Ec, *Klebsiella pneumonia*: Kp, *Pseudomonas aeruginosa*: Pa, *Proteus vulgaris*: Pv, *Salmonella typhimirium*: St, *Yersinia pseudotuberculosis*: Yp, *Enterobacter cloaceae*: Ecl. ID, diameter in (mm) of the inhibition zone around the disks (6 mm) impregnated with sample. MIC: Minimal inhibitory concentration as μg/mL. Ka: kanamycine were used as positive reference at 300 μg/disk (Sigma). Kanamycine (μg/mL) was used as reference antibiotic in micro well dilution assay (Sigma). Ge: gentamycine.

oven. The mixture was heated using a fixed power of 350 W for15 min at 85°C. The reaction mixture was dissolved in methanol and filtered off. The extract was evaporated to leave a crude mixture, which was purified by column chromatography over basic silica (hexane–ethyl acetate, 3:1, 3:2, 1:1) to afford the pure corresponding products (**2** and **3**) in 55 and 30% yields, respectively. When we do the same reaction in higher experimental condition using with a power of 500 W for 30 min at 110°C, major product was 1,3′-diazaflavone (60% yield).

2-(Pyridin-3-yl)-2,3-dihydroquinolin-4(1H)-one, 2. Bright yellow amorphous solid, see the physicochemical, ^{1}H and ^{13}C NMR data in Tables 1–4.

2-Pyridin-3-ylquinolin-4(1H)-one, 3. Yellow amorphous solid, see the physicochemical, ^{1}H and ^{13}C NMR data in Tables 1–4.

General procedure for synthesis of compounds 2a–j. 1,3′-Diazaflavanone (**2**) (∼0.45 mmol for each) and *n*-bromoalkanes (1-bromopentane, 1-bromohexane, 1-bromoheptane, 1-bromooctane, 1-bromononane, 1-bromodecane, 1-bromoundecane, 1-bromododecane, 1-bromotetradecane, and 1-bromopentadecane, 0.05 mol each) in acetonitrile (30 mL) were refluxed separately for 6–12 h [17–19]. On completion of the reaction, followed by TLC examination, the acetonitrile was removed using a rotary evaporator, and the residue was purified by column chromatography (column, length 30 cm, diameter 2 cm) on silica gel (25 g, Merck, 230–400 mesh). The column was eluted successively with the following solvents and solvent mixtures: ethyl acetate (30 mL), ethyl acetate-methanol (3:1, 20 mL and 3:2, 20 mL), and methanol (30 mL) then methanol–water (4:1, 30 mL). Fractions (5–10 mL each) were collected and monitored by analytical TLC. The desired dark-red amorphous solids, **2a–j**, were obtained from fractions 8–18 (yields are in Table 1).

General procedure for synthesis of compounds 3a–i. 1,3′-Diazaflavone (**3**) (∼0.45 mmol for each) and *n*-bromoalkanes (1-bromopentane, 1-bromohexane, 1-bromoheptane, 1-bromooctane, 1-bromononane, 1-bromodecane, 1-bromoundecane, 1-bromododecane, and 1-bromotetradecane, 0.50 mmol each) in acetonitrile (30 mL) using basic silica were refluxed separately for 6–12 h [17–19] On completion of the reaction, followed by TLC examination, the acetonitrile was removed using a rotary evaporator, and the residue was purified by column chromatography (column, length 30 cm, diameter 2 cm) on silica gel (25 g, Merck, 230–400 mesh). The column was eluted successively with the following solvents and solvent mixtures: ethyl acetate (30 mL), ethyl acetate-methanol (3:1, 20 mL and 3:2, 20 mL) and methanol (30 mL) then methanol–water (4:1, 30 mL). Fractions (5–10 mL each) were collected and monitored by analytical TLC. The desired amorphous solids, **3a–i**, were obtained from fractions 12–24 (yields are in Table 1).

1-Alkyl($C_{5-12,14,15}$)-3-(4-Oxo-1,2,3,4-tetrahydroquinolin-2-yl)pyridinium bromide, 2a–j. Yellow to red amorphous solids or oily, see the physicochemical, ^{1}H and ^{13}C NMR data in Tables 1–4.

1-Alkyl($C_{5-12,14}$)-3-(1-alkyl($C_{5-12,14}$)-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide, 3a–i. Yellow to red amorphous solids or oily, see the physicochemical, ^{1}H and ^{13}C NMR data in Tables 1–4.

Antimicrobial activity. ***Microbial strains.*** The compounds were tested individually against 11 species of Gram positive (*Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228)) and Gram negative (*Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 13315), *Salmonella typhimirium* (ATCC 14028), *Yersinia pseudotuberculosis* (ATCC 911), *Enterobacter cloaceae* (ATCC 13047)) bacteria. All bacterial strains were originally obtained from American Type Culture Collection (ATCC), and they were deposited at the Department of Biology, Faculty of Science, Karadeniz Technical University, Trabzon, Turkey.

Determination of the *in vitro* antimicrobial activity by the disc-diffusion method. The antimicrobial activity of the compounds was determined by means of the disc-diffusion method. Cultures of each bacteria were inoculated to Mueller–Hinton broth and incubated at 37°C for 16 h, then adjusted to $OD_{625} = 0.08–0.1$ (approximately $1 \times 10^{7}–1 \times 10^{8}$ CFU/mL). The bacterial suspensions (100 μL) was placed onto the surface of Mueller–Hinton agar in a 60-mm Petri dish and spread homogeneously with a Drigalski tip. Discs (6.0-mm diameter) were impregnated with 10 μL of methanol solution of the compounds **1–2**, **2a–j** (300 μg/disk) and placed on the surface of the agar containing each bacterium, which was incubated at 37° C for 24 h. The inhibition zones were measured with a caliper considering the total diameters. Similarly, each plate carried a blank disc containing 10 μL of methanol and an antibiotic disc (100 μg for gentamycin, 300 μg for kanamycine). Each experiment was performed in triplicate.

Microwell dilution assay. The MIC values were determined for the bacterial strains that were sensitive to the compounds **1–2**, **2a–j** in the disk-diffusion assay. The inocula of the bacterial strains were prepared from 12-h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The compound solutions were first diluted to the highest concentration (400 and 600 μg/mL) to be tested, and then serial twofold dilutions were made to obtain a concentration range from 12.5 to 400 μg/mL and 18.75 to 600 in 1-mL sterile test tubes containing Mueller-Hinton broth. The MIC values of the compounds **1–2**, **2a–j** against bacterial strains were determined based on a microwell dilution method [22]. The 96-well plates were prepared by dispensing 100 μL of Mueller-Hinton broth containing the inoculum into each well. One hundred microliters from the stock solutions of the compounds **1–2**, **2a–j** prepared at the 400 and 600 μg/mL concentration was added into the first wells. Then, 100 μL from the serial dilutions was transferred into the seven consecutive wells. The last well containing 200 μL of Mueller-Hinton broth without compound and with the inoculum on each strip was used as a negative control. The final volume in each well was 200 μL. Kanamycine at a concentration range of 500–15.6 μg/mL was prepared in Mueller-Hinton broth and used as a standard drug for positive control. The plate was covered with a sterile plate sealer. The contents of each well were incubated at 37°C temperatures for 24 h. Microbial growth in each medium was determined by reading the respective absorbance at 600 nm using the spectrophotometer (Molecular Devices, SpectraMax M2). The compounds tested in this study were screened twice against each organism.

CONCLUSIONS

The diazaflavanones and diazaflavones are analogous to flavanone and flavone-type natural compounds. In consideration of the pharmacological activities of flavanones/flavones and their analogs [1], 1,3′diazaflavanone (**2**), 1,3′-diazaflavone (**3**), and their *N*-alkyl (**2a–j**), *N*,*N*′-dialkyl **(3a–i)** derivatives were synthesized and tested against four G+ and seven G− bacteria. The synthesized compounds showed antimicrobial activity against only G+ bacteria. *N*-alkylated compounds **2c–j** and *N*,*N*′-dialkyl compounds **3a–c** were better antimicrobials. The alkyl substitution of the pyridyl ring of diazaflavanones brought about very high antimicrobial activity, which otherwise showed nonexisting (compounds **2** and **3**) activity. The alkyl substitution apparently makes these compounds better permeable by bacterial cell walls. In this respect, G+ bacteria were prone to the bactericidal or bacteriostatic action of the alkylated compounds **2c–j** and **3**a–c probably because of their more spacely structured cell wall peptidoglycan network and the lack of lipopolysaccharide outer layer.

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