Synthesis, antibacterial and antioxidant activities of new 1-alkyl-4-(1-alkyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromides

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · SEPTEMBER 2013

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2013.08.050 · Source: PubMed

European Journal of Medicinal Chemistry 69 (2013) 348–355

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, antibacterial and antioxidant activities of new 1-alkyl-4-(1-alkyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromides

Nuran Kahriman [a,*], Büşra Yaylı [a], Ayça Aktaş [a], Zeynep İskefiyeli [a], Fatih Şaban Beriş [b], Nurettin Yaylı [a]

[a] Department of Chemistry, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey
[b] Department of Biology, Faculty of Arts and Sciences, Recep Tayyip Erdoğan University, 53100 Rize, Turkey

ARTICLE INFO

Article history:
Received 6 May 2013
Received in revised form
25 August 2013
Accepted 31 August 2013
Available online 12 September 2013

Keywords:
Microwave
1,4′-Diazaflavone
Quinoline nucleus
N,N′-Dialkyl-1,4′-diazaflavonium bromide
Antibacterial activity
Antioxidant activity

ABSTRACT

New 1-alkyl-4-(1-alkyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromides (**3a**–**k**) were synthesized from 1,4′-diazaflavone [2-pyridin-4-ylquinolin-4(1*H*)-one] and evaluated for antibacterial and antioxidant activities. A rapid one-pot preparation of 1,4′-diazaflavone (**2**) was done from 2′-amino substituted chalcone (**1**) by intramolecular Michael addition using solvent-free microwave heating. New *N,N′*-dialkyl substituted (C_5–C_{15}) 1,4′-diazaflavonium bromides were synthesized from compound **2** with corresponding alkyl halides. Compounds **3a**–**k** were active against six bacteria (MIC: 7.8–500.0 μg/mL). They also showed good antioxidant activities in DPPH• scavenging (SC_{50}: 45–133 μg/mL) and ferric reducing/antioxidant power (14–141 μM TEAC) tests. The biological activities decreased as alkyl chain length increased. The reason behind the obvious negative effect of alkyl chain elongation is unclear and requires investigations about the intermolecular interactions of these pyridinium salts with bioassay components.

1. Introduction

Flavones are an important class of natural compounds belonging to the flavonoid group, which occur in fruits, nuts, seeds, vegetables, flowers, and barks [1–4]. They are an integral part of human diet and have a wide range of biological activities as antioxidant, anti-inflammatory, antibacterial, antitumor, DNA cleavage, anti-HIV, vasodilator, antiviral, antimutagenic, antiallergic, and anticancer [1,5–17]. Moreover, it is known that some flavonoids act as an inhibitor to release the histamine from human basophiles and as antifeedant [16]. Antibacterial activity of flavonoids has been widely studied. However, the action mechanism of the antibacterial flavonoids are largely unknown and thought to be through either of the following three routes: causing damage to cell membrane structure and function, inhibiting DNA and/or protein synthesis, and inhibiting energy metabolism [18,19].

1,4′-Diazaflavone [2-pyridin-4-ylquinolin-4(1*H*)-one] is an analog of flavones and azaflavones [15,20–24] with annular nitrogen atoms, and it contains a quinolin-4(1*H*)-one ring like many organic compounds such as dyes and pharmaceuticals [14,24,25]. Heterocyclic compounds containing quinoline nucleus have a special place in medicinal chemistry, and they are ubiquitous substructures related with biologically active natural products [26]. The quinoline compounds have been considered as good starting materials for the synthesis of new compounds with a wide spectrum of biological activities such as antimycobacterial, antiparasitical, antibacterial, cytotoxic, antineoplastic, antimalarial, antiviral, antitumor, immunomodulatory, antiangiogenesis, antileishmanial, antiarrhythmic, local anesthetic and anti-inflammatory behavior [26–31]. There are many synthetic procedures to prepare azaflavones including base or acid catalyzed reactions with corrosive reagents such as strong alkali or orthophosphoric acid [15,32,33]. There have been multiple recent reports, describing the synthesis of 2-aryl-4-quinolinones [34–36] including 1,4′-diazaflavone. 1,4′-diazaflavone has been synthesized with microwave irradiation from 2′-amino (*E*)-4″-aza-chalcone for the first time by our group [14,15].

Recently, there is a significant increase in the microwave-assisted solvent-free organic synthesis. Microwave synthesis offers advantages over conventional heating as providing short reaction times, improved yield, greater selectivity, cleaner reaction, and being environmentally friendly [14–16,31,37].

N-Alkyl and *N,N′*-dialkyl derivatives of azachalcones and diazaflavones have been reported to have a wide variety of biological activities such as antioxidant, antimicrobial, antituberculosis,

* Corresponding author. Tel.: +90 462 377 4276; fax: +90 462 325 31 96.
E-mail address: nuran_yayli@hotmail.com (N. Kahriman).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.08.050

antibacterial, and anti-inflammatory [15,22,38–41]. In our previous work, 1,4′-diazaflavone and their *N*-alkyl derivatives studied with a biological interest were reported to show good antimicrobial activity against only gram positive bacteria [14]. In view of our continuous interest on bioactive agents, we synthesized a series of *N*,*N*′-dialkyl (C_{5-15}) substituted-1,4′-diaza-flavonium bromides (**3a**–**k**) containing quinoline nucleus in this respect. We aimed to determine the influence of substitution with a second alkyl group attached to nitrogen in the central ring and the length of the alkyl chain in the *N*,*N*′-dialkyl substituent on the biological activities.

In this work, we prepared a new set of violet colored quinoline derivatives as *N*,*N*′-dialkyl bromide pigments (**3a**–**k**) from 1,4′-diazaflavone (**2**) synthesized with a microwave-assisted method. Then, all new compounds were investigated for their antibacterial and antioxidant activities.

2. Results and discussion

2.1. Synthesis

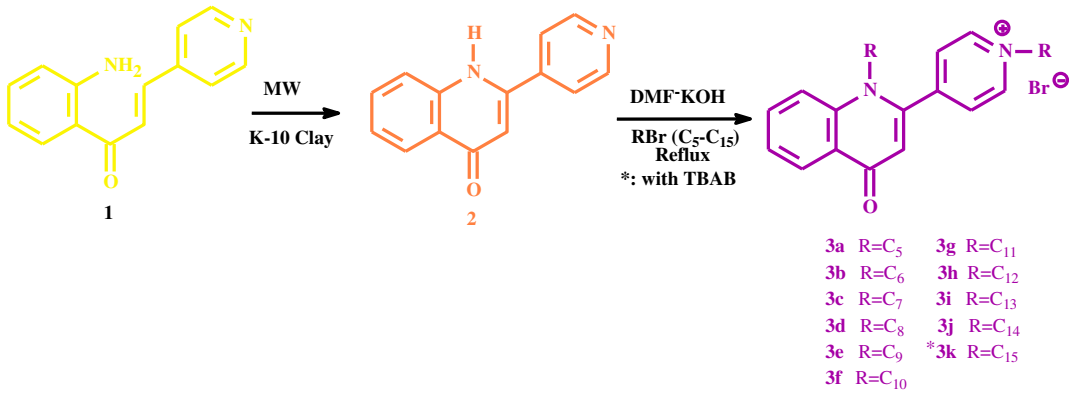
The synthesis of *N*,*N*′-dialkyl bromide pigments from 1,4′-diazaflavone was performed following the steps shown in reaction Scheme 1.

The 1,4′-diazaflavone (**2**) was obtained by intramolecular Michael addition using microwave heating in the absence of organic solvents as previously described [14]. Alkyl derivatives of flavonoids and chalconoids have attracted widespread interest because of their wide variety of biological activities [15,35,36,38–41]. Preparation of dialkyl derivatives (**3a**–**k**) was accomplished by reaction of 1,4′-diazaflavone with different alkyl halides using KOH in dimethyl formamide by reflux [15,35,36,38–41]. Compound **3k** was obtained in the presence of tetra-*n*-butyl ammonium bromide (TBAB) in addition to other reagents. The purity of all the synthesized compounds was checked by TLC after purification by column chromatography. We characterized all the newly synthesized compounds by spectroscopic data such as ^{1}H, ^{13}C, APT, $^{1}H-^{1}H$ COSY NMR, ACD-NMR, FT-IR, UV, LC-MS/MS, and elemental analysis. And all of them were in full agreement with the proposed structures. Based upon the above observations, the complete chemical shift assignments for **3a**–**k** was deduced to be 1-alkyl(C_{5-15})-4-(1-alkyl(C_{5-15})-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide.

2.2. Antibacterial screening

All of the synthesized compounds were tested against three Gram-positive and three Gram-negative bacteria in accordance with published protocols based on microdilution assay of antibacterial samples according to CLSI Standard Methods [42,43]. The results were compared with the standard drug ampicillin, which is one of the most commonly used broad-spectrum antibacterial agent against both Gram (+) and (−) bacteria. At the end of incubation, the effect of the newly synthesized compounds and ampicillin on the growth was determined (Table 1).

As can be seen from the table, all newly synthesized compounds showed antibacterial effect, ranging from good to moderate, with a minimum inhibitory concentration of 7.8–500.0 μg/mL in dimethyl sulfoxide, which is in contrast with the previous work with singly alkylated derivatives [14]. The second alkylation apparently affected antibacterial activity in the way that the compounds showed antibacterial activity against not only Gram (+) bacteria but also Gram (−) ones. In addition, all the compounds were active at test concentrations, another clear advantage of second alkyl group substitution. According to the MIC values, compounds **3a**–**f** showed similar high activity against both Gram (+) and Gram (−) bacteria. Compound **3b** showed the highest activity against all the test organisms. It had MIC values of 7.8 μg/mL to *Staphylococcus aureus* and *Pseudomonas aeruginosa*, 15.6, 31.3, and 62.5 μg/mL to *Klebsiella pneumonie*, *Bacillus subtilis*, *Enterococcus faecalis*, and *Proteus vulgaris*, respectively. The antibacterial activity of each compound was similar against Gram (+) and Gram (−) bacteria. This similarity was better observable when the means of the MIC values for each compound against Gram (+) and Gram (−) bacteria separately were plotted against the number of C in the alkyl chain in the series (Fig. 1). The trends of MIC value change as the C number in alkyl chain increased were quite same for Gram (+) and Gram (−) groups. It is easily understood from the graph that as the chain length increased, the MIC values increased, and thus the antibacterial activity decreased. Increase in the chain length apparently has a negative effect on antibacterial activity, which may be because of inaccessibility of the compounds through bacterial cell boundary. Because the trend and the values of MIC are very similar against Gram (+) and Gram (−) bacteria for each compound, the site of change in inaccessibility could be peptidoglycan layer or cell membrane, but probably not lipopolysaccharide layer, which exists only in Gram (−) bacteria. Another explanation of the similarity of the activities against Gram (+) and Gram (−) bacteria would be that as the chain length changes, the interaction of these compounds with the target bacterial molecule, the activity of which is critically affected resulting in cell growth inhibition, is changed. As well known the target molecules in bacteria for antibacterials can be various, such as enzymes of bacterial cell wall synthesis, transcription factors, cell membrane components, essential bacterial enzymes, components of protein synthesis machinery.



Scheme 1. The synthesis reaction of *N*,*N*′-dialkyl bromide pigments.

Table 1
In-vitro antibacterial activities of **3a**–**k**.

Compounds	Stock solution (μg/mL)	Microorganisms[a] and minimal inhibition concentration (MIC) value (μg/mL)					
		Gram-positive bacteria			Gram-negative bacteria		
		Bs	Ef	Sa	Kp	Pa	Pv
3a	5750	62.5	62.5	15.6	31.2	15.6	62.5
3b	2450	31.2	31.2	7.8	15.6	7.8	62.5
3c	3700	62.5	62.5	31.2	62.5	62.5	125
3d	5400	31.2	62.5	15.6	31.2	31.2	31.2
3e	2500	62.5	62.5	31.2	31.2	62.5	31.2
3f	5200	62.5	125.0	62.5	62.5	31.2	62.5
3g	5150	125.0	125.0	62.5	125.0	62.5	31.2
3h	3250	250.0	125.0	125.0	125.0	125.0	125.0
3i	6750	250.0	250.0	250.0	250.0	250.0	250.0
3j	7000	500.0	250.0	500.0	500.0	500.0	500.0
3k	6500	500.0	250.0	500.0	250.0	500.0	500.0
DMSO[b]		NE	NE	NE	NE	NE	NE
PC[c] + amp[d]	100 μg/mL	–	–	–	–	–	–
PC		+	+	+	+	+	+

NE: not effective, "+": growth, "–": no growth.
[a] Bs: *Bacillus subtilis* ATCC 66333, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Kp: *Klebsiella pneumonie* ATCC 13883, Pa: *Pseudomonas aeruginosa* ATCC 27853, Pv: *Proteus vulgaris* ATCC 13315.
[b] DMSO: dimethyl sulfoxide.
[c] PC: positive control as broth medium without chemical component and antibiotic.
[d] amp: ampicillin.

2.3. Antioxidant activity

The antioxidant activity of flavonoids is largely affected by the substitutions, and the number and position of the hydroxyl groups are highly important [44–46]. However, even unsubstituted flavones would posses antioxidant activity due to the conjugated double bond structures. The unhydroxylated flavonoids containing alkyl chain substituents have been shown to have a decreasing profile in antioxidant activity measured by $ABTS^{\bullet+}$ radical scavenging assay [47]. Similar results were also observed with other structures containing aromatic rings [48,49]. However, the exact mechanism of antioxidant action is not known.

The synthesized azaflavonoids, containing alkyl chains, were tested for their antioxidant activity by using $DPPH^{\bullet}$ radical scavenging and ferric reducing/antioxidant power tests. The results of $DPPH^{\bullet}$ scavenging assay are given in Fig. 2 as SC_{50} values. All the compounds showed radical scavenging activity in the test concentration range. The starting compounds **1** and **2** showed slightly lower activity as compared to alkyl derivatives. Although there was not a consistent change in the activity as the alkyl chain length increased, the regression line fitted with Microsoft Excel for only compounds **3a**–**k** in order to show the change in activity as alkyl chain length increases showed an increasing trend from 5 C to 15 C, which means the radical scavenging activity decreases with increasing the number of carbons in the alkyl substituent.

The results of the second antioxidant test method are shown in Fig. 3 as TEAC values, which were obtained from the calibration graph prepared with Trolox as the Trolox concentration giving same absorbance values as the test sample. The higher value of TEAC means higher antioxidant capacity. All the test compounds were active in FRAP test. The activity of the starting compounds **1** and **2** were in close to the average of that of alkyl derivatives. The regression line drawn with Microsoft Excel for compounds **3a**–**k** fitted better in the case of FRAP test, and the decrease in antioxidant activity was more consistent as alkyl chain length increased from 5 C to 15 C, which also shows that the antioxidant activity decreases

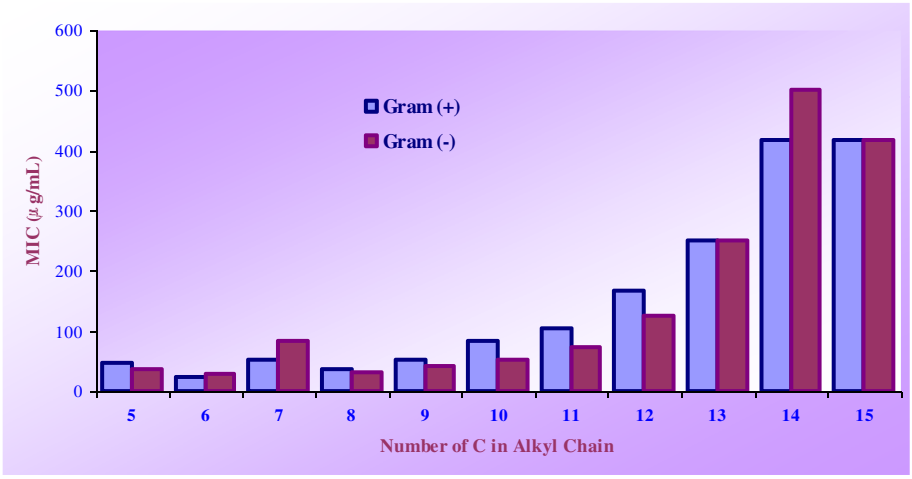


Fig. 1. The effect of alkyl chain length on antibacterial activity against Gram (+) and Gram (–) bacteria depicted as average of the MIC values for each compound against the three bacteria tested in each group.

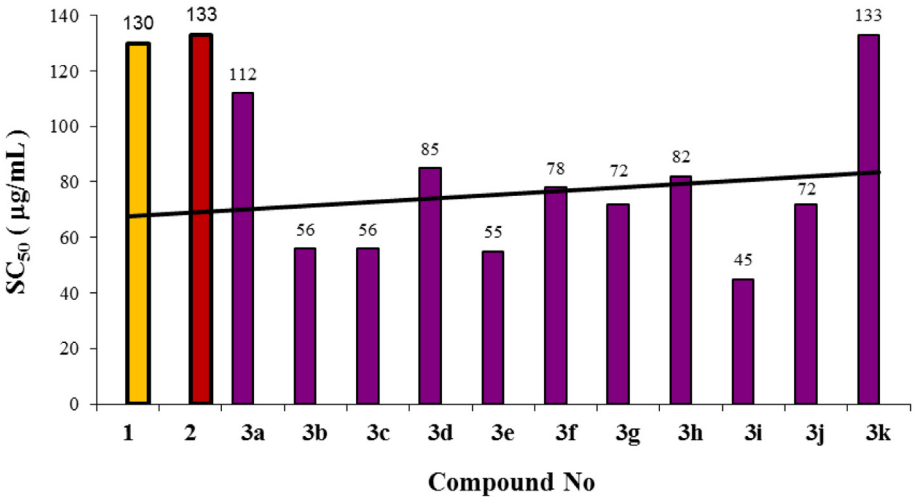


Fig. 2. SC_{50} (µg/mL) values of the compounds; the line drawn by linear regression in Microsoft Excel to show the trend of the SC_{50} change for only the compounds **3a**–**k** as the alkyl chain length increases.

with increasing the number of carbons in the alkyl substituent. Thus, the similar trends in the results of the two antioxidant tests suggest that chain length in the alkyl substituent has an inverse correlation with antioxidant activity.

3. Conclusion

In conclusion, a new series of 1-alkyl-4-(1-alkyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromides (**3a**–**k**) were synthesized, and these newly synthesized heterocyclic alkyl compounds, especially compounds **3a**–**f**, exhibited good antibacterial activities similarly against the Gram (+) and Gram (−) bacteria tested. The highest antibacterial activity among all compounds was observed with compound **3b**, especially against *S. aureus* and *P. aeruginosa*. The increasing trend of MIC values as alkyl chain length decreased showed a clear negative effect of increasing the number of carbons in alkyl substituent on antibacterial activity especially with 10 C or longer alkyls. Similarly, the antioxidant activity of the alkyl derivatives decreased as chain length increased in both antioxidant test methods. The elongation of alkyl chain has a detrimental effect on the biological activities of these pyridinium salts. All the compounds were active in antibacterial and antioxidant tests.

4. Experimental

4.1. Material

2-Aminoacetophenone, 4-pyridine carbaldehyde, bromoalkanes (C_{5-15}), potassium hydroxide and tetra-*n*-butyl ammonium bromide (TBAB) were purchased from Aldrich/Fluka and used without further purification. 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) radical and 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) were purchased from Sigma. The solvents (chloroform, *n*-hexane, dimethyl formamide, ethyl acetate, methanol, dimethyl sulfoxide, and diethyl ether) used were either of analytical grade or bulk solvents distilled before use. Thin-layer chromatography (TLC) and column chromatography were carried out on Merck precoated 60 Kieselgel F_{254} analytical aluminum acidic plates and silica gel 60 (0.040–0.063 mm), respectively.

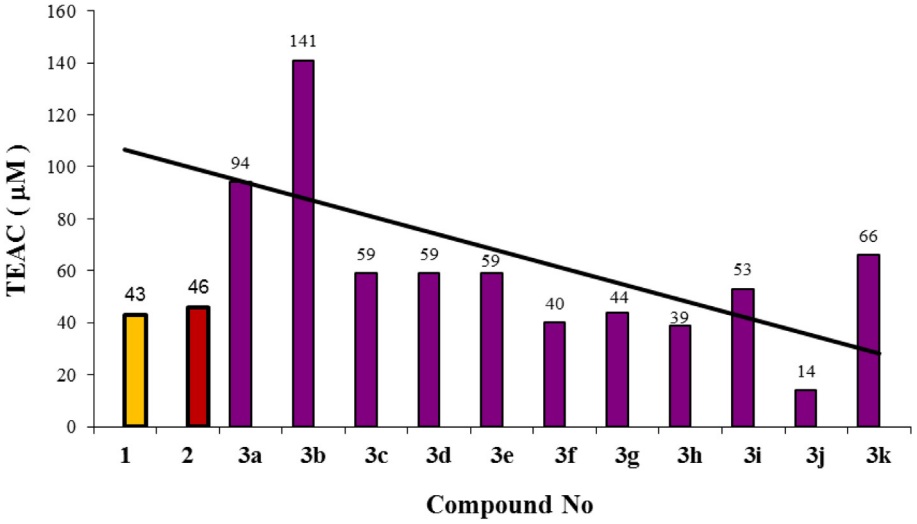


Fig. 3. FRAP test results of the compounds as TEAC values (TEAC: Trolox equivalent antioxidant capacity); the line drawn by linear regression in Microsoft Excel to show the trend of the TEAC change for only the compounds **3a**–**k** as the alkyl chain length increases.

4.2. Equipment

Milestone microwave oven was used for microwave included reaction. NMR spectra were recorded on a Varian Mercury NMR at 200 MHz in $CDCl_3$. NMR data assignment 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. The elemental analyses were performed on a Costech ECS 4010 instrument. Infrared spectra were obtained with a Perkin–Elmer 1600 FT-IR (4000–400 cm^{-1}) spectrometer. Melting points were determined by using a Thermo-var apparatus fitted with a microscope and are uncorrected. UV–Vis absorbance measurements and spectral analyses were carried out on a Unicam UV2-100 at 25 °C.

4.3. Methods

The known compounds **1** and **2** [14] were prepared according to the literature [14,15].

4.3.1. General procedure for synthesis of compounds **3a**–**k**

1,4′-Diazaflavone (**2**) (~0.68 mmol for each) was dissolved in 5 mL of DMF. Stoichiometric amount of KOH was added to the reaction flask. *n*-Bromoalkanes (1-bromopentane, 1-bromohexane, 1-bromoheptane, 1-bromooctane, 1-bromononane, 1-bromodecane, 1-bromoundecane, 1-bromododecane, 1-bromotridecana, 1-bromotetradecane, and 1-bromopentadecane, 1.50 mmol each) were added after conversion of dark orange colored mixture to dark green-brown. Also, catalytic amount of TBAB was used for compound **3k** because of unmixed phases. Then, all solutions were refluxed separately for 6–12 h [15]. On completion of the reaction, followed by TLC examination, the mixture 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: hexane (30 mL), ether (30 mL), ether-ethyl acetate (1:1, 50 mL), ethyl acetate (30 mL) and ethyl acetate-methanol (8:1, 90 mL) and methanol (30 mL). Fractions (5–10 mL each) were collected and monitored by analytical TLC. The desired violet oils or amorphous solids **3a**–**k** were obtained from fractions 6–13.

*4.3.1.1. 1-Pentyl-4-(1-pentyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide (**3a**).* Yield 80%. Violet oil. R_f (AcOEt/methanol 3:1) 0.71. FT-IR (NaCl, $CHCl_3$, cm^{-1}): 3026, 2956, 2930, 2860, 1716, 1634, 1611, 1578, 1466, 1353, 1171, 1129, 901, 753. UV–Vis λ_{max}($CHCl_3$)/nm (log ε): 552 (3.9), 342 (4.4), 256 (4.1). ^1H NMR (200 MHz, $CDCl_3$): 9.0 (d, J = 6.2, 2H–C(3′/5′)); 8.72 (d, J = 6.2, 2H–C(2′/6′)); 7.63 (d, J = 7.4, H–C(5)); 7.56 (t, J = 7.8, H–C(6)); 6.99 (t, J = 7.8, H–C(7)); 6.93 (d, J = 7.8, H–C(8)); 6.54 (s, H–C(3)); 4.80 (t, J = 7.0, 2H–C(1‴)); 3.91 (t, J = 7.2, 2H–C(1″)); 2.03 (m, 2H–C(2‴)); 1.73 (m, 2H–C(2″)); 1.37–1.23 (series of m. CH_2, 8H–C(3″–4″/3‴–4‴)); 0.91 (m, 6H, H–C(5″/5‴)). ^{13}C NMR (50 MHz, $CDCl_3$): 185.5 (C(4)); 152.4 (C(2)); 150.6 (C(1′)); 142.7 (C(3′/5′)); 142.5 (C(9)); 137.5 (C(7)); 126.8 (C(5)); 125.4 (C(6)); 121.4 (C(2′/6′)); 120.1 (C(10)); 109.6 (C(8)); 106.2 (C(3)); 60.5 (C(1‴)); 42.7 (C(1″)), 31.3, 28.1, 27.9, 26.5, 22.4, 22.3 (C(2″–4″/2‴–4‴)); 13.8 (C (5‴)); 13.7 (C(5″)). Poz. LC-MS/MS: 363 (100, [M – $^{79}Br/^{81}Br]^+$); 364 (40, [M – $^{79}Br/^{81}Br$ + 1]$^+$); 293 (35, [M – ^{79}Br – R + 1]$^+$); 292 (15, [M – $^{79}Br/^{81}Br$ – R + 1]$^+$); 365 (10, [M – $^{79}Br/^{81}Br$ + 2]$^+$). Anal. cal. for $C_{24}H_{31}N_2OBr$ (443.43): C: 65.01, H: 7.05 N: 6.32 found: C: 65.08, H: 7.10, N: 6.39.

*4.3.1.2. 1-Hexyl-4-(1-hexyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide (**3b**).* Yield 72%. Violet oil. R_f (AcOEt/methanol 3:1) 0.75. FT-IR (NaCl, $CHCl_3$, cm^{-1}): 3042, 2956, 2928, 2857, 1719, 1636, 1613, 1578, 1467, 1355, 1172, 1130, 752. UV–Vis λ_{max}($CHCl_3$)/nm (log ε): 552 (3.9), 342 (4.5), 256 (4.3). ^1H NMR (200 MHz, $CDCl_3$): 8.94 (d, J = 6.6, 2H–C(3′/5′)); 8.71 (d, J = 6.2, 2H–C(2′/6′)); 7.64 (d, J = 7.8, H–C(5)); 7.55 (t, J = 7.8, H–C(6)); 7.00 (t, J = 7.8, H–C(7)); 6.92 (d, J = 7.8, H–C(8)); 6.50 (s, H–C(3)); 4.76 (t, J = 7.0, 2H–C(1‴)); 3.89 (t, J = 7.0, 2H–C(1″)); 2.00 (m, 2H–C(2‴)); 1.71 (m, 2H–C(2″)); 1.38–1.33 (series of m. CH_2, 12H–C(3″–5″/3‴–5‴)); 0.88 (m, 6H, H–C(6″/6‴)). ^{13}C NMR (50 MHz, $CDCl_3$): 185.6 (C(4)); 152.5 (C(2)); 150.7 (C(1′)); 142.7 (C(3′/5′)); 142.6 (C(9)); 137.6 (C(7)); 126.9 (C(5)); 125.5 (C(6)); 121.5 (C(2′/6′)); 120.2 (C(10)); 109.6 (C(8)); 106.2 (C(3)); 60.7 (C(1‴)); 42.9 (C(1″)), 31.6, 31.5, 31.2, 31.1, 28.1, 26.8, 25.7, 22.3 (C(2″–5″/2‴–5‴)); 14.0 (C(6‴)), 13.9 (C(6″)). Poz. LC-MS/MS: 391 (100, [M – $^{79}Br/^{81}Br]^+$); 392(40, [M – $^{79}Br/^{81}Br$ + 1]$^+$); 393(20, [M – $^{79}Br/^{81}Br$ + 2]$^+$); 307 (8, [M – $^{79}Br/^{81}Br$ – R + 1]$^+$). Anal. cal. for $C_{26}H_{35}N_2OBr$ (471.48): C: 66.24, H: 7.48 N: 5.94; found: 66.19, H: 7.53, N 5.96.

*4.3.1.3. 1-Heptyl-4-(1-heptyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide (**3c**).* Yield 75%. Violet oil. R_f (AcOEt/methanol 3:1) 0.78. FT-IR (NaCl, $CHCl_3$, cm^{-1}): 3020, 2952, 2925, 2855, 1735, 1681, 1632, 1614, 1575, 1466, 1364, 1169, 1134, 891, 750. UV–Vis λ_{max}($CHCl_3$)/nm (log ε): 552 (4.0), 342 (4.5), 256 (4.3), 240 (4.0). ^1H NMR (200 MHz, $CDCl_3$): 8.93 (d, J = 6.4, 2H–C(3′/5′)); 8.71 (d, J = 6.6, 2H–C(2′/6′)); 7.63 (d, J = 7.4, H–C(5)); 7.55 (t, J = 8.0, H–C(6)); 6.99 (t, J = 7.4, H–C(7)); 6.91 (d, J = 8.0, H–C(8)); 6.46 (s, H–C(3)); 4.75 (t, J = 6.8, 2H–C(1‴)); 3.88 (t, J = 7.0, 2H–C(1″)); 2.00 (m, 2H–C(2‴)); 1.75 (m, 2H–C(2″)); 1.37–1.23 (series of m. CH_2, 16H–C(3″–6″/3‴–6‴)); 0.86 (m, 6H, H–C(7″/7‴)). ^{13}C NMR (50 MHz, $CDCl_3$): 185.5 (C(4)); 152.5 (C(2)); 150.6 (C(1′)); 142.8 (C(3′/5′)); 142.5 (C(9)); 137.5 (C(7)); 126.9 (C(5)); 125.5 (C(6)); 121.5 (C(2′/6′)); 120.1 (C(10)); 109.6 (C(8)); 106.3 (C(3)); 60.7 (C(1‴)); 42.8 (C(1″)), 31.8, 31.6, 31.4, 29.1, 28.7, 27.1, 26.8, 25.9, 22.5, 22.4 (C(2″–6″/2‴–6‴)); 14.0 (C (7‴)); 13.9 (C(7″)). Poz. LC-MS/MS: 419 (100, [M – $^{79}Br/^{81}Br]^+$); 420 (35, [M – $^{79}Br/^{81}Br$ + 1]$^+$); 421 (20, [M – $^{79}Br/^{81}Br$ + 2]$^+$); 321 (8, [M – $^{79}Br/^{81}Br$ – R + 1]$^+$). Anal. cal. for $C_{28}H_{39}N_2OBr$ (499.53): C: 67.32, H: 7.87 N: 5.61; found: 67.37, H: 7.91, N 5.64.

*4.3.1.4. 1-Octyl-4-(1-octyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide (**3d**).* Yield 83%. Violet amorphous solid. M.p.: 89–92 °C. R_f (AcOEt/methanol 3:1) 0.78. FT-IR (NaCl, $CHCl_3$, cm^{-1}): 3022, 2952, 2923, 2854, 1732, 1680, 1632, 1698, 1578, 1467, 1338, 1168, 1132, 894, 750. UV–Vis λ_{max}($CHCl_3$)/nm (log ε): 552 (4.1), 342 (4.6), 258 (4.3). ^1H NMR (200 MHz, $CDCl_3$): 8.96 (d, J = 6.2, 2H–C(3′/5′)); 8.71 (d, J = 6.0, 2H–C(2′/6′)); 7.62 (d, J = 7.0, H–C(5)); 7.54 (t, J = 7.4, H–C(6)); 6.97 (t, J = 7.6, H–C(7)); 6.91 (d, J = 8.2, H–C(8)); 6.50 (s, H–C(3)); 4.77 (t, J = 6.6, 2H–C(1‴)); 3.89 (t, J = 7.0, 2H–C(1″)); 1.99 (m, 2H–C(2‴)); 1.74 (m, 2H–C(2″)); 1.38–1.24 (series of m. CH_2, 20H–C(3″–7″/3‴–7‴)); 0.85 (m, 6H, H–C(8″/8‴)). ^{13}C NMR (50 MHz, $CDCl_3$): 185.5 (C(4)); 152.5 (C(2)); 150.6 (C(1′)); 142.8 (C(3′/5′)); 142.5 (C(9)); 137.5 (C(7)); 126.9 (C(5)); 125.5 (C(6)); 121.4 (C(2′/6′)); 120.2 (C(10)); 109.6 (C(8)); 106.3 (C(3)); 60.7 (C(1‴)); 42.8 (C(1″)), 31.7, 31.6, 31.4, 29.5, 29.1, 28.9, 27.1, 26.8, 26.0, 22.5 (C(2″–7″/2‴–7‴)); 14.0 (C(8″)); 14.0 (C (8‴)). Poz. LC-MS/MS: 447 (100, [M – $^{79}Br/^{81}Br]^+$); 448 (30, [M – $^{79}Br/^{81}Br$ + 1]$^+$); 449 (15, [M – $^{79}Br/^{81}Br$ + 2]$^+$); 335 (5, [M – $^{79}Br/^{81}Br$ – R + 1]$^+$). Anal. cal. for $C_{30}H_{43}N_2OBr$ (527.59): C: 68.30, H: 8.22 N: 5.31; found: 68.34, H: 8.27, N 5.33.

*4.3.1.5. 1-Nonyl-4-(1-nonyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide (**3e**).* Yield 65%. Violet amorphous solid. M.p.: 108–111 °C. R_f (AcOEt/methanol 3:1) 0.81. FT-IR (NaCl, $CHCl_3$, cm^{-1}): 3042, 2957, 2923, 2854, 1730, 1693, 1636, 1612, 1577, 1466, 1352, 1168, 1129, 892, 750. UV–Vis λ_{max}($CHCl_3$)/nm (log ε): 552 (3.7), 342 (4.4), 256 (4.3). ^1H NMR (200 MHz, $CDCl_3$): 8.93 (d, J = 6.0,

2H–C(3′/5′)); 8.70 (d, J = 6.0, 2H–C(2′/6′)); 7.62 (d, J = 7.4, H–C(5)); 7.54 (t, J = 7.8, H–C(6)); 6.97 (t, J = 7.4, H–C(7)); 6.91 (d, J = 8.2, H–C(8)); 6.48 (s, H–C(3)); 4.78 (t, J = 6.8, 2H–C(1‴)); 3.88 (t, J = 7.2, 2H–C(1″)); 1.97 (m, 2H–C(2‴)); 1.73 (m, 2H–C(2″)); 1.38–1.23 (series of m. CH_2, 24H–C(3″–8″/3‴–8‴)); 0.85 (m, 6H, H–C(9″/9‴)). ^{13}C NMR (50 MHz, $CDCl_3$): 185.5 (C(4)); 152.5 (C(2)); 150.6 (C(1′)); 142.8 (C(3′/5′)); 142.5 (C(9)); 137.6 (C(7)); 126.9 (C(5)); 125.5 (C(6)); 121.5 (C(2′/6′)); 120.1 (C(10)); 109.6 (C(8)); 106.3 (C(3)); 60.7 (C(1‴)); 42.8 (C(1″)), 31.7, 29.4, 29.2, 29.1, 29.0, 28.8, 27.1, 26.8, 26.1, 22.5 (C(2″–8″/2‴–8‴)); 14.0 (C(9″)); 14.0 (C (9‴)). Poz. LC-MS/MS: 447 (100, [M – ^{79}Br/^{81}Br]$^+$); 448 (30, [M – ^{79}Br/^{81}Br + 1]$^+$); 449 (15, [M – ^{79}Br/^{81}Br + 2]$^+$); 335 (5, [M – ^{79}Br/^{81}Br – R + 1]$^+$). Anal. cal. for $C_{32}H_{47}N_2OBr$ (555.64): C: 69.17, H: 8.53, N: 5.04; found: C: 69.26, H: 8.61, N: 5.13.

*4.3.1.6. 1-Decyl-4-(1-decyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide (**3f**).* Yield 73%. Violet amorphous solid. M.p.: 110–113 °C. R_f (AcOEt/methanol 3:1) 0.81. FT-IR (NaCl, $CHCl_3$, cm^{-1}): 3031, 2957, 2922, 2853, 1731, 1692, 1634, 1575, 1465, 1338, 1287, 1127, 896, 750. UV–Vis λ_{max}($CHCl_3$)/nm (log ε): 552 (4.0), 344 (4.5), 256 (4.2). ^{1}H NMR (200 MHz, $CDCl_3$): 8.91 (d, J = 6.0, 2H–C(3′/5′)); 8.72 (d, J = 5.8, 2H–C(2′/6′)); 7.65 (d, J = 7.4, H–C(5)); 7.56 (t, J = 7.4, H–C(6)); 6.99 (t, J = 7.8, H–C(7)); 6.92 (d, J = 8.0, H–C(8)); 6.51 (s, H–C(3)); 4.76 (t, J = 7.0, 2H–C(1‴)); 3.90 (t, J = 7.2, 2H–C(1″)); 2.00 (m, 2H–C(2‴)); 1.74 (m, 2H–C(2″)); 1.37–1.23 (series of m. CH_2, 28H–C(3″–9″/3‴–9‴)); 0.86 (m, 6H, H–C(10″/10‴)). ^{13}C NMR (50 MHz, $CDCl_3$): 185.6 (C(4)); 152.5 (C(2)); 150.7 (C(1′)); 142.7 (C(3′/5′)); 142.6 (C(9)); 137.6 (C(7)); 126.9 (C(5)); 125.6 (C(6)); 121.5 (C(2′/6′)); 120.2 (C(10)); 109.7 (C(8)); 106.3 (C(3)); 60.8 (C(1‴)); 42.9 (C(1″)), 32.2, 31.8, 29.5, 29.3, 29.2, 29.1, 29.0, 27.1, 26.9, 26.1, 25.8, 22.6 (C(2″–9″/2‴–9‴)); 14.0 (C(10″)); 14.0 (C (10‴)). Poz. LC-MS/MS: 503 (100, [M – ^{79}Br/^{81}Br]$^+$); 504 (38, [M – ^{79}Br/^{81}Br + 1]$^+$); 505 (10, [M – ^{79}Br/^{81}Br + 2]$^+$); 363 (8, [M – ^{79}Br/^{81}Br – R + 1]$^+$). Anal. cal. for $C_{34}H_{51}N_2OBr$ (583.70): C: 69.96, H: 8.81 N: 4.80; found: C: 69.88, H: 8.91, N: 4.72.

*4.3.1.7. 1-Undecyl-4-(1-undecyl-4-oxo-1,4-dihydroquinolin-2-yl) pyridinium bromide (**3g**).* Yield 80%. Violet amorphous solid. M.p.: 112–115 °C. R_f (AcOEt/methanol 3:1) 0.81. FT-IR (NaCl, $CHCl_3$, cm^{-1}): 3019, 2952, 2921, 2853, 1728, 1698, 1632, 1596, 1576, 1466, 1337, 1286, 1130, 894, 751. UV–Vis λ_{max}($CHCl_3$)/nm (log ε): 550 (3.9), 346 (4.3), 256 (4.2). ^{1}H NMR (200 MHz, $CDCl_3$): 8.95 (d, J = 6.0, 2H–C(3′/5′)); 8.71 (d, J = 6.0, 2H–C(2′/6′)); 7.63 (d, J = 7.0, H–C(5)); 7.57 (t, J = 7.4, H–C(6)); 6.97 (t, J = 7.6, H–C(7)); 6.92 (d, J = 8.0, H–C(8)); 6.46 (s, H–C(3)); 4.78 (t, J = 6.8, 2H–C(1‴)); 3.87 (t, J = 7.0, 2H–C(1″)); 1.99 (m, 2H–C(2‴)); 1.73 (m, 2H–C(2″)); 1.38–1.23 (series of m. CH_2, 32H–C(3″–10″/3‴–10‴)); 0.86 (m, 6H, H–C(11″/11‴)). ^{13}C NMR (50 MHz, $CDCl_3$): 185.5 (C(4)); 152.4 (C(2)); 150.6 (C(1′)); 142.8 (C(3′/5′)); 142.7 (C(9)); 137.5 (C(7)); 126.8 (C(5)); 125.5 (C(6)); 121.5 (C(2′/6′)); 120.1 (C(10)); 109.6 (C(8)); 106.2 (C(3)); 60.7 (C(1‴)); 42.8 (C(1″)), 32.2, 31.8, 29.4, 29.2, 29.0, 27.1, 26.8, 26.0, 25.8, 22.6 (C(2″–10″/2‴–10‴)); 14.0 (C(11″)); 14.0 (C (11‴)). Poz. LC-MS/MS: 531 (100, [M – ^{79}Br/^{81}Br]$^+$), 532 (25, [M – ^{79}Br/^{81}Br+1]$^+$), 533 (10, [M – ^{79}Br/^{81}Br + 2]$^+$); 377 (8, [M – ^{79}Br/^{81}Br – R + 1]$^+$). Anal. cal. for $C_{36}H_{55}N_2OBr$ (611.78): C:70.68, H: 9.06 N: 4.58; found: C: 70.47, H: 9.19, N: 4.56.

*4.3.1.8. 1-Dodecyl-4-(1-dodecyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide (**3h**).* Yield 85%. Violet amorphous solid. M.p.: 115–118 °C. R_f (AcOEt/methanol 3:1) 0.81. FT-IR (NaCl, $CHCl_3$, cm^{-1}): 3020, 2920, 2852, 1727, 1680, 1632, 1576, 1467, 1340, 1291, 1171, 1132, 896, 751. UV–Vis λ_{max}($CHCl_3$)/nm (log ε)): 552 (4.3), 344 (4.8), 256 (4.6). ^{1}H NMR (200 MHz, $CDCl_3$): 8.89 (d, J = 6.6, 2H–C(3′/5′)); 8.72 (d, J = 6.4, 2H–C(2′/6′)); 7.64 (d, J = 7.4, H–C(5)); 7.55 (t, J = 7.4, H–C(6)); 6.99 (t, J = 7.6, H–C(7)); 6.94 (d, J = 8.2, H–C(8)); 6.50 (s, H–C(3)); 4.75 (t, J = 7.2, 2H–C(1‴)); 3.90 (t, J = 7.4, 2H–C(1″)); 2.03 (m, 2H–C(2‴)); 1.75 (m, 2H–C(2″)); 1.37–1.22 (series of m. CH_2, 36H–C(3″–11″/3‴–11‴); 0.87 (m, 6H, H–C(12″/12‴)). ^{13}C NMR (50 MHz, $CDCl_3$): 185.6 (C(4)); 151.5 (C(2)); 150.7 (C(1′)); 142.7 (C(3′/5′)); 142.6 (C(9)); 137.6 (C(7)); 126.9 (C(5)); 125.6 (C(6)); 121.5 (C(2′/6′)); 120.2 (C(10)); 109.7 (C(8)); 106.3 (C(3)); 60.8 (C(1‴)); 42.9 (C(1″)), 32.2, 31.8, 29.5, 29.3, 29.0, 27.1, 26.9, 26.1, 25.8, 22.6 (C(2″–11″/2‴–11‴)); 14.1 (C(12″)); 14.1 (C (12‴)). Poz. LC-MS/MS: 559 (100, [M – ^{79}Br/^{81}Br]$^+$); 560 (25, [M – ^{79}Br/^{81}Br + 1]$^+$); 561 (14, [M – ^{79}Br/^{81}Br + 2]$^+$); 391 (5, [M – ^{79}Br/^{81}Br – R + 1]$^+$). Anal. cal. for $C_{38}H_{59}N_2OBr$ (639.80): C:71.34, H: 9.29, N: 4.38; found: C: 71.29, H: 9.35, N: 4.29.

*4.3.1.9. 1-Tridecyl-4-(1-tridecyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide (**3i**).* Yield 72%. Violet amorphous solid. M.p.: 123–126 °C. R_f (AcOEt/methanol 3:1) 0.83. FT-IR (NaCl, $CHCl_3$, cm^{-1}): 3029, 2959, 2919, 2851, 1735, 1694, 1633, 1575, 1466, 1339, 1171, 1132, 894, 750. UV–Vis λ_{max}($CHCl_3$)/nm (log ε): 552 (4.2), 344 (4.7), 258 (4.4). ^{1}H NMR (200 MHz, $CDCl_3$): 8.95 (d, J = 6.0, 2H–C(3′/5′)); 8.71 (d, J = 5.8, 2H–C(2′/6′)); 7.62 (d, J = 7.8, H–C(5)); 7.55 (t, J = 7.0, H–C(6)); 6.99 (t, J = 7.4, H–C(7)); 6.92 (d, J = 8.2, H–C(8)); 6.49 (s, H–C(3)); 4.79 (t, J = 7.0, 2H–C(1‴)); 3.89 (t, J = 7.4, 2H–C(1″)); 1.99 (m, 2H–C(2‴)); 1.74 (m, 2H–C(2″)); 1.38–1.21 (series of m. CH_2, 40H–C(3″–12″/3‴–12‴)); 0.88 (m, 6H, H–C(13″/13‴)). ^{13}C NMR (50 MHz, $CDCl_3$): 185.5 (C(4)); 152.5 (C(2)); 150.6 (C(1′)); 142.8 (C(3′/5′)); 142.5 (C(9)); 137.5 (C(7)); 126.8 (C(5)); 125.5 (C(6)); 121.5 (C(2′/6′)); 120.1 (C(10)); 109.6 (C(8)); 106.2 (C(3)); 60.7 (C(1‴)); 42.8 (C(1″)), 32.21, 31.8, 31.7, 29.5, 29.4, 29.3, 29.0, 27.1, 26.8, 26.0, 22.6 (C(2″–12″/2‴–12‴)); 14.0 (C(13″)); 14.0 (C (13‴)). Poz. LC-MS/MS: 587 (100, [M – ^{79}Br/^{81}Br]$^+$); 588 (35, [M – ^{79}Br/^{81}Br + 1]$^+$); 589 (20, [M – ^{79}Br/^{81}Br + 2]$^+$); 405 (10, [M – ^{79}Br/^{81}Br – R + 1]$^+$). Anal. cal. for $C_{40}H_{63}N_2OBr$ (667.86): C: 71.94, H: 9.51, N: 4.19; found: C:71.95, H: 9.56, N: 4.22.

*4.3.1.10. 1-Tetradecyl-4-(1-tetradecyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide (**3j**).* Yield 80%. Violet amorphous solid. M.p.: 124–128 °C. R_f (AcOEt/methanol 3:1) 0.81. FT-IR (NaCl, $CHCl_3$, cm^{-1}): 3033, 2954, 2919, 2851, 1711, 1694, 1633, 1573, 1544, 1465, 1341, 1174, 1130, 901, 750, 719. UV–Vis λ_{max}($CHCl_3$)/nm (log ε): 552 (4.1), 344 (4.6), 258 (4.3). ^{1}H NMR (200 MHz, $CDCl_3$): 8.96 (d, J = 5.8, 2H–C(3′/5′)); 8.72 (d, J = 5.8, 2H–C(2′/6′)); 7.62 (d, J = 8.0, H–C(5)); 7.54 (t, J = 7.8, H–C(6)); 6.98 (t, J = 7.4, H–C(7)); 6.92 (d, J = 8.2, H–C(8)); 6.51 (s, H–C(3)); 4.79 (t, J = 6.8, 2H–C(1‴)); 3.89 (t, J = 7.4, 2H–C(1″)); 1.99 (m, 2H–C(2‴)); 1.75 (m, 2H–C(2″)); 1.38–1.23 (series of m. CH_2, 44H–C(3″–13″/3‴–13‴)); 0.88 (m, 6H, H–C(14″/14‴)). ^{13}C NMR (50 MHz, $CDCl_3$): 185.5 (C(4)); 152.5 (C(2)); 150.6 (C(1′)); 142.8 (C(3′/5′)); 142.5 (C(9)); 137.6 (C(7)); 126.9 (C(5)); 125.5 (C(6)); 121.5 (C(2′/6′)); 120.2 (C(10)); 109.7 (C(8)); 106.4 (C(3)); 60.8 (C(1‴)); 42.9 (C(1″)), 31.9, 31.7, 29.6, 29.5, 29.3, 29.1, 27.2, 26.9, 26.1, 22.6 (C(2″–13″/2‴–13‴)); 14.1 (C(14″)); 14.1 (C (14‴)). Poz. LC-MS/MS: 615 (100, [M – ^{79}Br/^{81}Br]$^+$); 616 (35, [M – ^{79}Br/^{81}Br + 1]$^+$); 617 (18, [M – ^{79}Br/^{81}Br + 2]$^+$); 419 (5, [M – ^{79}Br/^{81}Br – R + 1]$^+$). Anal. cal. for $C_{42}H_{67}N_2OBr$ (695.91): C: 72.49, H: 9.70, N: 4.03; found: C: 72.53, H: 9.75, N: 4.09.

*4.3.1.11. 1-Pentadecyl-4-(1-pentadecyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromide (**3k**).* Yield 68%. Violet amorphous solid. M.p.: 127–130 °C. R_f (AcOEt/methanol 3:1) 0.78. FT-IR (NaCl, $CHCl_3$, cm^{-1})): 3040, 2959, 2919, 2851, 1725, 1694, 1634, 1573, 1465, 1342, 1174, 1130, 751. UV–Vis λ_{max}($CHCl_3$)/nm (log ε): 552 (3.8), 344 (4.2), 256 (4.2). ^{1}H NMR (200 MHz, $CDCl_3$): 8.88 (d, J = 5.8, 2H–C(3′/5′)); 8.68 (d, J = 6.0, 2H–C(2′/6′)); 7.62 (d, J = 7.4, H–C(5)); 7.55 (t, J = 7.6, H–C(6)); 6.99 (t, J = 7.4, H–C(7)); 6.92 (d, J = 8.4, H–C(8)); 6.46 (s, H–C(3)); 4.73 (t, J = 7.0, 2H–C(1‴)); 3.87 (t, J = 7.6, 2H–C(1″)); 1.98 (m, 2H–C(2‴)); 1.71 (m, 2H–C(2″)); 1.38–1.23 (series of m. CH_2,

48H–C(3″–14″/3‴–14‴)); 0.87 (m, 6H, H–C(15″/15‴)). ^{13}C NMR (50 MHz, $CDCl_3$): 185.6 (C(4)); 152.5 (C(2)); 150.7 (C(1′)); 142.7 (C(3′/5′)); 142.6 (C(9)); 137.6 (C(7)); 126.9 (C(5)); 125.6 (C(6)); 121.6 (C(2′/6′)); 120.2 (C(10)); 109.7 (C(8)); 106.2 (C(3)); 60.8 (C(1‴)); 42.9 (C(1″)), 31.9, 31.7, 29.6, 29.3, 29.1, 29.0, 27.2, 26.9, 26.1, 24.2, 22.7 (C(2″–14″/2‴–14‴)); 14.1 (C(15″)); 14.1 (C(15‴)). Poz. LC-MS/MS: 643 (100, $[M - {}^{79}Br/{}^{81}Br]^+$); 644 (28, $[M - {}^{79}Br/{}^{81}Br + 1]^+$); 645 (20, $[M - {}^{79}Br/{}^{81}Br + 2]^+$); 433 (5, $[M - {}^{79}Br/{}^{81}Br - R + 1]^+$. Anal. cal. for $C_{42}H_{71}N_2OBr$ (723.96): C: 72.00, H: 9.88, N: 3.87; found: C: 72.03, H: 9.90, N: 3.89.

4.4. Antibacterial activity

The screening of the susceptibility spectra of different bacterial strains to the compounds was performed by the quantitative assay of minimal inhibitory concentration (MIC, μg/mL) based on liquid medium serial microdilutions [42,43]. The MIC assays were performed in LB medium at pH 7.2. The stock solutions of the compounds were prepared in dimethyl sulfoxide (DMSO). The dilution series of the chemical compounds to be tested were prepared from 1000 to 7.8 μg/mL concentrations in 100 μL medium. The broth cultures were incubated at 37.0 ± 1 °C for 18–24 h. DMSO, LB medium with or without antibiotic, and ampicillin, were used as solvent control, positive, and negative controls, respectively. The MIC was taken to be the last well in the dilution series that did not exhibit growth as determined on the basis of turbidity.

The determination of minimum inhibitory concentration [43] was done with the three Gram-positive bacteria *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, and *E. faecalis* ATCC 29212 and the three Gram-negative bacteria *K. pneumonie* ATCC 13883, *P. aeruginosa* ATCC 27853, and *P. vulgaris* ATCC 13315. The bacteria were inoculated into Luria broth medium containing 1% tryptone, 0.5% yeast extract, and 0.5% sodium chloride. The pH of the medium was adjusted to 7.2 and incubated at 37 °C for 18–24 h. The optical density of the bacteria from mid-log phase of growth was measured at 600 nm and diluted in fresh medium so as to get an optical density of 0.004 (corresponding to 5×10^5 colony forming units/mL).

4.5. Antioxidant activity tests

Two or more antioxidant test methods with different strategies or chemistries are generally utilized in antioxidant activity determinations because differences appear in many cases between the results of different assays due to different reaction mechanisms and kinetics, varying effects of solvents, existence of sterical issues, and varying effects of temperature, pH, and matrix components. In the current study, two of the most widely used antioxidant test methods were used for the determination of the antioxidant capacities of the synthesized compounds. DPPH$^\bullet$ radical scavenging test has been used extensively for various types of samples including synthetic compounds [50]. Similarly, ferric reducing/antioxidant power (FRAP) method has been utilized in many investigations with synthetic organics. To overcome solubility issues of the compounds when the solutions are mixed with FRAP reagent, the original method [51,52] has been modified to contain methanol in 3:2 ratio in water instead of using water as solvent in the preparation of FRAP reagent.

4.5.1. DPPH$^\bullet$ scavenging test

DPPH$^\bullet$ radical (2,2-diphenyl-1-picrylhydrazyl) is stable in solid form, and it is, thus, widely used in antioxidant research [50]. A 750 μL 50 μM methanolic solution of DPPH$^\bullet$ was mixed with 750 μL sample solutions of five different concentrations (6.25, 12.50, 25.00, 50.00, and 100.00 μg/mL final test concentration). The contents were vortex-mixed and incubated for 50 min at room temperature. The absorbances measured at 517 nm were plotted against sample concentrations, and SC_{50} values (μg/mL) were determined from the graphs as the sample concentration reducing DPPH$^\bullet$ concentration to half of its initial value. Reagent blank and solvent control tests were also made, and the results were used in the construction of the graphs. Lower SC_{50} values indicate higher radical scavenging potential.

4.5.2. Ferric reducing/antioxidant power (FRAP) test

FRAP test method was first developed as antioxidant test method by using $FeSO_4$ solutions [50], and later improved by using TPTZ (2,4,6-tris(2-pyridyl)-*s*-triazine) as complexing agent for ferrous ions produced from ferric ions in the medium as a result of reducing/antioxidant action of the antioxidant sample [44]. FRAP results show indirectly the total reducing potential of the sample. The method utilizes the measurement of absorbance at 595 nm caused by Fe^{2+} – TPTZ complex.

The method was modified to prevent insolubility problems of the compounds with aqueous media of the original FRAP reagent, changing the reagent solvent to methanol:water (3:2) mixture. FRAP reagent was freshly prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in 10:1:1 ratio, respectively. Methanolic Trolox solutions of five different concentration (1000–500–250–125–62.5 μM) were used in parallel with the compound solutions of 1 mg/mL concentration in the FRAP test. Sample and Trolox solutions (100 μL) were mixed with FRAP reagent (3 mL), vortexed, and allowed to stand for 20 min at room temperature, and the absorbance was read at 595 nm against water blank. Reagent blank, containing sample solvent and FRAP reagent, and sample blanks, containing sample and FRAP solvent, were also tested, and the sum of the absorbance of these two measurements was subtracted from the absorbance mean obtained by triplicate measurements with each sample.

Absorbance values for Trolox were used to construct calibration curve, plotting [Trolox] vs absorbance. The ferric reducing/antioxidant power of the samples were expressed as Trolox Equivalent Antioxidant Capacity (TEAC), calculated as the Trolox concentration from the graph corresponding to the absorbance observed with sample. Higher TEAC value means higher FRAP, i.e. higher antioxidant capacity.

Acknowledgments

This study was supported by grants from Karadeniz Technical University and the Scientific and Technological Research Council of Turkey (TÜBİTAK-109T388).

References

[1] G.W. Kabalka, A.R. Mereddy, Tetrahedron Lett. 46 (2005) 6315–6317.
[2] V.M. Malikov, M.P. Yuldashev, Chem. Nat. Compd. 38 (2002) 358–406.
[3] C.W. Huck, C.G. Huber, K.H. Ongania, G.K. Bonn, J. Chromatogr. A 870 (2000) 453–462.
[4] T. Nagao, F. Abe, J. Kinjo, H. Okabe, Biol. Pharm. Bull. 7 (2002) 875–879.
[5] M.J. Chan-Bacab, L.M. Pena-Rodriguez, Nat. Prod. Rep. 18 (2001) 674–688.
[6] J.B. Harborne, Nat. Prod. Rep. 16 (1999) 509–523.
[7] F. Perez-Vizcaino, M. Ibarra, A.L. Cogolludo, J. Duarte, F. Zaragoza-Arnaez, L. Moreno, G. Lopez-Lopez, J.J. Tamargo, Pharmacol. Exp. Ther. 301 (2002) 66–71.
[8] N. Matsuo, K. Yamada, K. Yamashia, K. Shoji, M. Mori, M. Sugano, In Vitro Cell Dev. Biol. 32 (1996) 340–344.
[9] A. Jain, M.C. Martin, N. Praveen, N.U. Khan, J.H. Parish, S.M. Hadi, Phytother. Res. 13 (1999) 609–612.
[10] J. Yamada, Y. Tomita, Biosci. Biotechnol. Biochem. 58 (1994) 2197–2200.
[11] G.M. Shivji, E. Zielinska, S. Kondo, H. Mukhtar, D.N. Sander, J. Invest. Dermatol. 106 (1996) 787–792.
[12] (a) H.X. Xu, S.F. Lee, Phytother. Res. 15 (2001) 39–43;
(b) J.M.T. Hamiltonmiller, Antimicrob. Agents Chemother. 39 (1995) 2375–2377.
[13] (a) C. Han, Cancer Lett. 114 (1997) 153–158;
(b) D.F. Birt, S. Hendrich, W. Wang, Pharmacol. Ther. 90 (2001) 157–177.

[14] N. Yaylı, O. Üçüncü, N. Yaylı, E. Demir, Z. Demirba&gbreve, Turk. J. Chem. 32 (2008) 785–975.
[15] N. Kahriman, N. Yılmaz İskender, M. Yücel, N. Yaylı, E. Demir, Z. Demirbağ, J. Heterocycl. Chem. 49 (2012) 71–79.
[16] M.J. Menezes, S. Manjrekar, V. Pai, R.E. Patre, S.G. Tilve, Indian J. Chem. 48B (2009) 1311–1314.
[17] J.A. Seijas, M.P. Vazques-Tato, R. Carballido-Reboredo, J. Org. Chem. 70 (2005) 2855–2858.
[18] T.P.T. Cushnie, A.J. Lamb, Int. J. Antimicrob. Agents 26 (2005) 343–356.
[19] K. Ulanowska, A. Tkaczyk, G. Konopa, G. Węgrzyn, Arch. Microbiol. 184 (2006) 271–278.
[20] J.B. Harborne, The Flavonoids: Advances in Research, Chapman&Hall, London, 1988.
[21] N. Ahmed, J.E. van Lier, Tetrahedron Lett. 48 (2007) 13–15.
[22] M.L. Edwars, D.M. Stemeric, J.S. Sabol, K.A. Diekema, R.J. Dinerstein, J. Med. Chem. 37 (1994) 4357–4362.
[23] N. Ahmed, J.E. van Lier, Tetrahedron Lett. 47 (2006) 2725–2729.
[24] M.F. Springsteel, L.J.V. Galietta, T. Ma, K. By, G.O. Berger, H. Yang, C.W. Dicus, W. Choung, C. Quan, A.A. Shelat, R.K. Guy, A.S. Verkman, M.J. Kurth, M.H. Nantz, Bioorgan. Med. Chem. 11 (2003) 4113–4120.
[25] M.J. Sandelier, P. DeShong, Org. Lett. 9 (2007) 3209–3212.
[26] C.M. Melendez Gómez, V.V. Kouznetsov, M.A. Sortino, S.L. Álvarez, S.A. Zacchino, Bioorg. Med. Chem. 16 (2008) 7908–7920.
[27] M.A. Hussein, A.N. Kafafy, S.G. Abdel-Moty, O.M.F. Abou-Ghadir, Acta Pharm. 59 (2009) 365–382.
[28] B. Insuasty, D. Becerra, J. Quiroga, R. Abonia, M. Nogueras, J. Cobo, Eur. J. Med. Chem. 60 (2013) 1–9.
[29] Y. Xia, Z.Y. Yang, P. Xia, K.B. Bastow, Y. Tachibana, S.C. Kuo, E. hamel, T. Hackl, K.H. Lee, J. Med. Chem. 41 (1998) 1155–1162.
[30] Z.Y. An, Y.Y. Yan, D. Peng, T.M. Qu, J.H. Tan, S.L. Huang, L.K. an, L.Q. Gu, Z.S. Huang, Eur. J. Med. Chem. 45 (2010) 3895–3903.
[31] S.J. Song, S.J. Cho, D.K. Park, T.W. Kwon, S.A. Jenekhe, Tetrahedron Lett. 44 (2003) 255–257.
[32] C.P. Jones, K.W. Anderson, S.L. Buchwald, J. Org. Chem. 72 (2007) 7968–7973.
[33] S. Tollari, S. Cenini, F. Ragaini, L. Cassar, J. Chem. Soc. Chem. Commun. 15 (1994) 1741–1742.
[34] J.K. Son, S. Kim III, Y. Jahng, Heterocycles 55 (2001) 1981–1986.
[35] A.C. Annigeri, S. Siddappa, Monatsh. Chem. 96 (1965) 625–630.
[36] A.C. Annigeri, S. Siddappa, Indian J. Chem. 2 (1964) 413–415.
[37] R. Martinez-Palou, J. Mex. Chem. Soc. 51 (2007) 252–264.
[38] Z. Nowakowska, E. Wyrzykiewicz, B. Kedzia, Il. Farm. 56 (2001) 325–329.
[39] Z. Nowakowska, E. Wyrzykiewicz, B. Kedzia, Il. Farm. 57 (2002) 657–661.
[40] N. Yaylı, M. Küçük, O. Üçüncü, A. Yaşar, N. Yaylı, Ş.A. Karaoğlu, J. Photochem. Photobiol. A 188 (2007) 161–168.
[41] N. Yaylı, O. Üçüncü, A. Yaşar, M. Küçük, N. Yaylı, E. Akyüz, Ş.A. Karaoğlu, Turk. J. Chem. 30 (2006) 505–514.
[42] Approved Standart, CLSI document M07-A8Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, eighth ed., Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, 19087-21898 USA, 2009.
[43] M. Kaspady, V.K. Narayanaswamy, M. Raju, G.K. Rao, Lett. Drug Des. Discov. 6 (2009) 21–28.
[44] K.E. Heim, A.R. Tagliaferro, D.J. Bobilya, J. Nutr. Biochem. 13 (2002) 572–584.
[45] O. Farkas, J. Jakus, K. Héberger, Molecules 9 (2004) 1079–1088.
[46] D.P. Belsare, S.C. Pal, A.A. Kazi, R.S. Kankate, S.S. Vanjari, Int. J. ChemTech Res. 2 (2010) 1080–1089.
[47] C.B. An, D. Li, Y.Z. Bu, S. Wang, E.H. Zhang, P. Wang, X.C. Ai, J.P. Zhang, L.H. Skibsted, J. Agr. Food Chem. 59 (2011) 12652–12657.
[48] G. Chen, Y. Wang, X. Hao, S. Mu, Q. S, Chem. Cent. J. 5 (2011) 1–5.
[49] N. Takahashi, T. Ohba, T. Yamauchi, K. Higashiyama, Bioorgan. Med. Chem. 14 (2006) 6089–6096.
[50] M. Cuendet, P. Hostettmann, O. Potterat, Helv. Chim. Acta 80 (1997) 1144–1152.
[51] M. Oyaizu, Jpn. J. Nutr. 44 (1986) 307–315.
[52] I.F.F. Benzie, J.J. Strain, Anal. Biochem. 239 (1996) 70–76.