European Journal of Medicinal Chemistry 66 (2013) 345–354

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Antimicrobial activity of imidazo[1,5-*a*]quinoxaline derivatives with pyridinium moiety

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ARTICLE INFO

Article history:
Received 31 March 2013
Received in revised form
8 May 2013
Accepted 25 May 2013
Available online 7 June 2013

Dedicated to the 75th anniversary of Professor Vladimir Savich Reznik.

Keywords:
Imidazo[1,5-*a*]quinoxalines
Pyridinium salt
Bacteriostatic activity
Fungistatic activity

ABSTRACT

3-Phenyl(methyl)-5-alkyl-1-(pyridin-3-yl)imidazo[1,5-*a*]quinoxalin-4-ones (**2a**–**f**) and their *N*-alkylpyridinium salts (**3a**–**o**), including 1,*n*-bis{3-(3-phenylimidazo[1,5-*a*]quinoxalin-4(5*H*)-on-1-yl)pyridinium}alkane dibromides (**4a**–**d**, **5**, **6**) have been synthesized. It has been established that the antimicrobial properties of imidazo[1,5-*a*]quinoxaline derivatives are connected with the presence of various alkyl substituents in the position 1 of the pyridine ring and in the position 5 of the imidazo[1,5-*a*]quinoxaline system. Chlorides and iodides are more active towards bacteria than fungi. Compounds **3d**, **3e**, **3m** and **3n** showed an effective bacteriostatic activity. Compound showed not only well defined bacteriostatic activities but also good fungistatic activities, with the MIC values comparable with the reference drugs.

Toxicity of more effective (imidazo[1,5-*a*]quinoxalin-4-on-1-yl)-1-pyridinium halides was examined in mice.

1. Introduction

Heterocyclic systems containing quinoxaline moiety exhibit important biological activities. Many of them possess anticancer [1,2], antiviral [3], antibacterial [4], antiinflammatory [5] properties and serve as inhibitor of enzymes by catalyzing the transport of the phosphate group from ATP (kinase inhibitor) [6]. Quinoxalines are used as antibiotics [7,8], DNA-binding agents [9], building blocks in the synthesis of anion receptors [10], etc. Imidazoquinoxaline is one of the important classes of heterocycles that are often found in biologically active and pharmacologically useful agents such as Dazoquinast (antiallergic), FG 10571, NNC 14-0571, Panadiplon, U-78875 anxiolytic (benzodiazepine receptor partial agonist), U-8044 (antidepressant, anxiolytic), U-97775 (anxiolytic ($GABA_A$ receptor ligand)) and LU 73068 (anticonvulsant glycine/NMDA) but not in the NMDA receptor antagonist (Fig. 1) [11].

Some of the derivatives of imidazoquinoxalines are used in the treatment of central nervous system ailments [12,13] such as anticonvulsant, anxiolytic, hypnotic, and sedative/hypnotic properties [13–15]. Imidazoquinoxalines are proved to be useful as GABAa receptor ligands or prodrugs and selective GABA agonists, antagonists or inverse agonists of GABA receptors [16–20]. They are also used for the treatment of anxiety, sleep disorders, seizure disorders or for memory enhancement [20]. Many of these compounds contain substituents in various positions of 1,2,4-oxadiazol-3-yl [3,8,9,11,12,15], 1,2,4-oxadiazol-5-yl [14], 1,2-oxazol-3-yl [13], 2-oxazol-5-yl [13], imidazol-1-yl [21–23], pyrrolidin-1-yl [15,23] and morpholin-4-yl [21,23] moieties. This affects the manifestation of one or the other kind of properties. There is only one patent with the imidazo[1,5-*a*]quinoxaline derivatives containing a pyridinyl moiety in their structure as useful in treatment of central nervous system diseases such as psychosis and also in treatment of obesity, type 2 diabetes, metabolic syndrome, glucose intolerance, and pain [24].

The introduction of a pyridyl moiety with the free nitrogen atom into imidazoquinoxalines is assumed to greatly enhance their possibilities in synthesis and also in searching for physiologically active compounds. We assumed that due to the additional binding with the biotarget the efficiency of a specific moiety connected with imidazo[1,5-*a*]quinoxaline and pyridyl units would dramatically increase. The latter can be caused by the more rigid framework of the bis-heterocyclic systems with two quaternized nitrogen atoms in comparison with their mono counterparts. The bis-

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http://dx.doi.org/10.1016/j.ejmech.2013.05.038

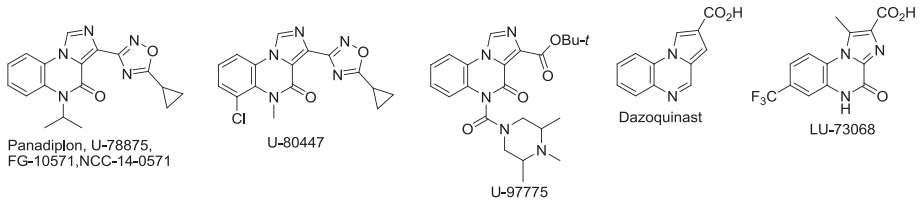


Fig. 1. Some quinoxaline derivatives of pharmacolical interest.

heterocyclic system involves complementary with the biotarget, particularly with the bis-heterocyclic systems with two pyridyl moieties and can exhibit activity due to the macrocyclic framework as a specific target.

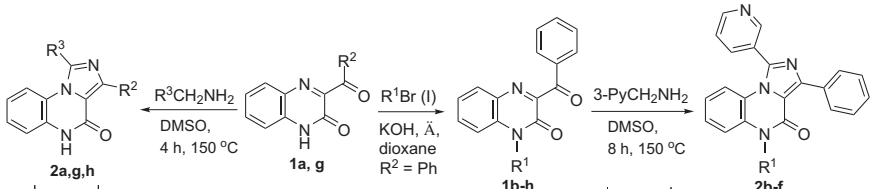
To our knowledge, in spite of advances in imidazo[1,5-*a*]quinoxalines chemistry, the biological activity for these compounds have so far not been investigated. The possible explanation might be due to the insolubility of almost all the known imidazo[1,5-*a*] quinoxalines in water, which prevents testing their biological properties. A wide screening of various kinds of biological activity is possible for imidazoquinoxalines of this type.

It is known that quaternary ammonium salts in combination with lipophilic residues display nonspecific activity through interaction with cell walls and cell membranes of microorganisms. The use of organic cations as disinfectants in agriculture, food processing industry and clinics is particularly important because they possess a high antibacterial activity and a broad spectrum of antimicrobial activity. Taking into account of the above points and considering the imidazoquinoxaline framework as a specific target to interfere with the main biochemical pathways (cell wall synthesis, DNA and RNA synthesis, protein formation) we have prepared a series of 1-(pyridin-3-yl)imidazo[1,5-*a*]quinoxalin-4-ones, and quaternized them by benzyl and alkyl halides. This preliminary communication demonstrates the *in vitro* antibacterial and antifungal activities of the imidazo[1,5-*a*]quinoxalinones and their toxicity, which have so far not been studied. We have also synthesized and tested a new type of imidazo[1,5-*a*]quinoxalinaphane with two 1-(pyridin-3-yl)imidazo[1,5-*a*]quinoxalin-4-one units and two spacers. One of them is the decamethylene fragment which connects the N4 and N4′ of the carbamoyl function of pyrazinone ring systems, and another one is the *m*-xylylene fragment which connects N and N′ of the pyrimidine rings. Besides, we tested the 1-(pyridin-3-yl)imidazo[1,5-*a*]quinoxalin-4-ones with the structural features of the examined bis-compounds and imidazo [1,5-*a*]quinoxalinaphane. The imidazo[1,5-*a*]quinoxalin-4-ones are subjected to screening in an attempt to determine the units responsible for antimicrobial activity and to evaluate the importance of ring-closure for efficiency. The objective of our study is to generate novel bioactive compounds and to optimize the structure to display the potency. As new infectious diseases appear time to time, it has become especially important to search for absolutely new agents for their remedy.

2. Chemistry

We have recently reported the synthesis of imidazo[1,5-*a*] quinoxalines by the reaction of 3-aroylquinoxalin-2(1*H*)-ones and their *N*-alkyl analogues with benzylamines in DMSO. The reaction proceeds through an intermediate formation of *N*-(α-quinoxalinylbenzylydene)benzylamine, which when subjected to oxidative cyclocondensation gives imidazo[1,5-*a*]quinoxalines [25]. The same synthetic protocol was used for the preparation of pyridyl containing imidazo[1,5-*a*]quinoxalin-4-ones. The use of 3-acylquinoxalin-2(1*H*)-ones **1a**, **g** and *N*-alkyl derivatives **1b**–**h**, and aminomethylpyridines make it possible to develop fundamentally new methods of imidazoannulation and lead to 1-pyridylimidazo[1,5-*a*]quinoxalin-4(5*H*)-ones **2g**, **h** and their *N*-alkylated derivatives **2b**–**f** (Scheme 1). The 1-pyridylimidazo[1,5-*a*]quinoxalin-4(5*H*)-ones **2a**, **g**, **h** were synthesised from ketones **1a**, **b**, **g** and picolylamines under heating conditions in DMSO in good yields [25]. In the cases of **c**–**f** a modified procedure involving alkylation of compound **1a** with alkylbromide was used with subsequent imidazoannulation of the crude *N*-bromalkyl derivatives **1b**–**f** with the 3-picolylamine. The products **2b**–**f** were readily isolated in pure form by filtration and the overall yield of the two-stage process **1a** → **1b**–**f** → **2b**–**f** is 44–57% (Scheme 1).

The reaction of 1-(pyridin-3-yl)imidazo[1,5-*a*]quinoxalin-4(5*H*)-ones with various types of alkyl halides gave rise to water-soluble derivatives **3**, **4**, **6** with a quaternized nitrogen atom in the pyridine ring system. Reaction conditions were optimised to obtain compounds **4** in which they were precipitated in their pure form from the reaction mixture. The synthesis of compounds **4a**, **b**, containing the NH group, were carried out in DMF, whereas synthesis of compounds **4c**, **d** with *N*-alkylated fragment in acetonitrile (Table 1).



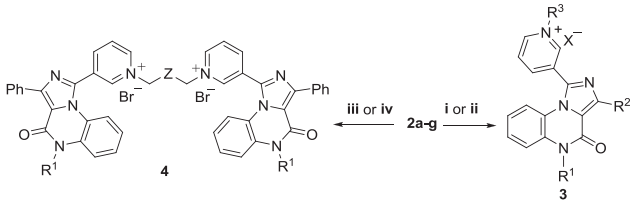
2	R^2	R^3
a	Ph	3-Py
g	Me	3-Py
h	Ph	2-Py

1, 2	R^1	Yield[b],
b	Me	76 (65)[a]
c	Pr	51 (32)[a]
d	C_6H_{13}	57 (42)[a]
e	C_9H_{19}	51 (35)[a]
f	$C_{18}H_{37}$	44 (30)[a]

[a] drop out of the reaction mixture in analytically pure form
[b] in case **c-f** for 2 steps (alkylation and annulation)

Scheme 1. The synthesis of imidazo[1,5-*a*]quinoxalin-4-ones **2a**–**h**.

Table 1
The synthesis of 3-(imidazo[1,5-*a*]quinoxalin-4-on-1-yl)pyridinium halides **3a**–**o** and **4a**–**d**.



Entry	Compound	R^1	R^2	R^3	Z	X	Yield, %
1	**3a**	H	Ph	Bn	–	Cl	87
2	**3b**	Me	Ph	Bn	–	Cl	71
3	**3c**	Pr	Ph	Bn	–	Cl	70
4	**3d**	C_6H_{13}	Ph	Bn	–	Cl	79
5	**3e**	C_9H_{19}	Ph	Bn	–	Cl	69
6	**3f**	$C_{18}H_{37}$	Ph	Bn	–	Cl	74
7	**3g**	H	Me	Bn	–	Cl	64
8	**3h**	H	Ph	Bu	–	I	95
9	**3i**	H	Ph	C_6H_{13}	–	Br	87
10	**3j**	C_6H_{13}	Ph	C_6H_{13}	–	I	72
11	**3k**	C_9H_{19}	Ph	C_6H_{13}	–	I	69
12	**3l**	C_6H_{13}	Ph	C_9H_{19}	–	Br	65
13	**3m**	C_6H_{13}	Ph	C_9H_{19}	–	I	68
14	**3n**	C_9H_{19}	Ph	C_9H_{19}	–	Br	64
15	**3o**	C_9H_{19}	Ph	C_9H_{19}	–	I	75
16	**4a**	H	–	–	$(CH_2)_2$	Br	51
17	**4b**	H	–	–	$(CH_2)_4$	Br	55
18	**4c**	C_6H_{13}	–	–	*m*-C_6H_4	Br	72
19	**4d**	C_9H_{19}	–	–	*m*-C_6H_4	Br	68

Reagents and conditions: (i) 1.5 equiv of R^3X, DMF, 120 °C, 16 h (**3a**,**b**,**g**,**h**,**i**); (ii) 1.5 equiv of R^3X, CH_3CN, reflux, 24 h (**3c**–**f**, **3j**–**o**); (iii) 0.5 equiv of $BrCH_2ZCH_2Br$, DMF, 120 °C, 16 h (for **4a**,**b**); (iv) 0.5 equiv of *m*-xylene dibromide, CH_3CN, reflux, 24 h (**4c**,**d**).

Macrocycle **6** was obtained under similar conditions like **4c**–**d** but from a considerably more diluted (40 times or 1 mg/mL or ~1 mmol/L) solution (Scheme 2).

3. Biological results and discussion

The antibacterial and antifungal activity of the imidazo[1,5-*a*] quinoxaline derivatives with pyridinium salts were investigated *in vitro* against several pathogenic representative Gram-negative bacteria (*Pseudomonas aeruginosa* 9027 and *Escherichia coli* F-50), Gram-positive bacteria (*Staphylococcus aureus* 209p and *Bacillus cereus* 8035), and pathogenic fungi (*Aspergillus niger* BKMF-1119, *Trichophyton mentagrophytes*) and yeast (*Candida albicans* 885-653). The results are summarized in Tables 2–4.

The screened compounds exhibit bacteriostatic and fungistatic activity in the regions of 0.78–500 μg/mL and 0.97–500 μg/mL concentrations, respectively. As evident from antibacterial data, imidazo[1,5-*a*]quinoxalines **3a**, **b**, **j**, **k** are showing a slight bacteriostatic and fungistatic activity against Gram-positive bacteria *S. aureus* and *B. cereus* and pathogenic yeast *C. albicans* but are absolutely inactive towards Gram-negative bacteria *E. coli*, *P. aeruginosa* and moulds *A. niger* and *T. mentagrophytes*.

The imidazo[1,5-*a*] quinoxaline derivatives which have been studied can be divided into three groups depending on the counterions.

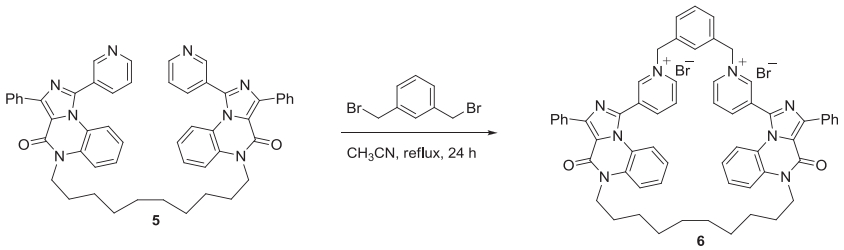
The first group contains chlorine as the counter-ion (**3a**–**g**), the second one contains bromine (**3i**, **l**, **n**) and the third one contains iodine (**3h**, **j**, **k**, **m**, **o**). It was established that the main criteria for evaluating the antimicrobial activity of imidazo[1,5-*a*]quinoxaline derivatives were the introduction of various substituents into position 1 of the pyridine ring and in position 5 of the imidazo[1,5-*a*] quinoxaline system and the presence of the charge in the molecule. This assumption was confirmed by the experiments determining the antimicrobial activity of hydrochlorides of compounds **2a** and **2h** [25] (Fig. 2), which represent uncharged molecules with unsubstituted nitrogen atoms at the position 1 of the pyridine ring and at position 5 of the imidazoquinoxaline system. From the data outlined in Table 2 it is evident that these compounds do not possess any antimicrobial activity against all the microorganisms used in the tests.

In the first group of imidazo[1,5-*a*]quinoxaline derivatives a comparative analysis of compounds with an unsubstituted nitrogen atom in the position 5 of the imidazo[1,5-*a*]quinoxaline system **3a**, with compounds containing a variety of alkyl groups in this position were carried out. As can be seen from the data in Table 2 the introduction of the methyl group in the position 5 does not affect the antimicrobial activity of **3b**. While the presence of the propyl (C_3H_7) group in **3c** leads to a sharp decrease in the fungistatic activity, as well as reduction of the bacteriostatic activity by two times against Gram-positive bacteria *S. aureus*. Compound **3d**, which contains the hexyl (C_6H_{13}) group in the position 5 is the best both in its bacteriostatic and its fungistatic activities in the first group. This compound has a low antibacterial activity against *S. aureus* as compared with the antibiotic Ciprofloxacin, but exhibits an inhibitory effect at 0.97 μg/ml against Gram-positive bacteria *S. aureus*, similar to the MIC of the antibiotic Ofloxacin, but much better than Norfloxacin (at MIC 2.42 μg/ml).

The increase in the lipophilicity of the carbon chain up to nonyl (C_9H_{19}) **3e** led to a decrease in the bacteriostatic activity by two times and increase in the fungistatic activity by two as compared with the compound **3d**. Introducing an even longer chain such as octadecanyl ($C_{18}H_{37}$) **3f** resulted in completely loss of the antimicrobial activity of imidazo[1,5-*a*]quinoxaline derivatives.

Replacement of the phenyl by methyl in the imidazo[1,5-*a*] quinoxaline derivative dramatically reduces the antimicrobial activity in the compound **3g**.

The second group of compounds containing hexyl (C_6H_{13}) and nonyl (C_9H_{19}) groups were studied. The most active compound was **3l** with the nonyl (C_9H_{19}) group in the position 1 of the pyridine ring and the hexyl (C_6H_{13}) group in the position 5 of the imidazo [1,5-*a*]quinoxaline system. The antimicrobial activity is lower than Ciprofloxacin and Clotrimazole but it possesses a bacteriostatic and fungistatic activity against Gram-positive bacteria and yeast, comparable to the known drugs – Ofloxacin and Amphotericin B.



Scheme 2. The synthesis of diimidazo[1,5-*a*]quinoxalinapyridinabenzenacyclophane **6**.

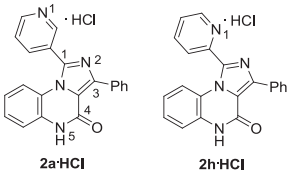


Fig. 2. The hydrochlorides of 1-(pyridin-3-yl)- (**2a**) and 1-(pyridin-4-yl)- (**2h**) imidazo[1,5-*a*]quinoxalin-4-ones without any alkyl groups at the position 1 of the pyridine ring and at the position 5 of the imidazoquinoxaline system.

The introduction of the nonyl (C_9H_{19}) group in the position 5 (compound **3n**) decreased its both bacteriostatic properties by 1.5–6 times, and the fungistatic factor by 50 times as compared with the compound **3l**.

Compound **3i**, with an unsubstituted nitrogen atom in the position 5 of the imidazoquinoxaline system ranks considerably below the compound **3l** containing various alkyl (C_6H_{13}, C_9H_{19}) groups and the compound **3n**, containing the same alkyl (C_9H_{19}) groups in its activity.

The analysis of the test results of the compounds of group 3 showed that the introduction of the butyl (C_4H_9) group in position 1 of the pyridine ring (compound **3h**) leads to a sharp decrease in the antimicrobial activity. The introduction of the hexyl (C_6H_{13}) group in the position 1 of the pyridine ring and in the position 5 of the imidazo[1,5-*a*]quinoxaline system (compound **3j**) leaded to the increase in the fungistatic activity against the yeast *C. albicans* by 16 times as compared with the compound **3i**, containing the hexyl (C_6H_{13}) group only in position 1 of the pyridine ring.

Compound **3m** exhibits a lower antimicrobial activity than the antibiotics Ciprofloxacin, but shows an inhibitory effect at 0.78 μg/ml against Gram-positive bacteria, similar to the MIC as the antibionic Ofloxacin, but much better then Norfloxacin (MIC at 2.42 μg/ml).

The compounds **3m** and **3o** have structures similar to those of compounds **3l** and **3n** from the second group. A comparative analysis of the data showed that the change of the counter-ion from bromine to iodine leads to a sharp decrease in the fungistatic properties of compounds **3m** and **3o** as compared with the **3l** and **3n**. Bacteriostatic properties vary slightly. The introduction of the hexyl (C_6H_{13}) group into position 1 of the pyridine ring and the nonyl (C_9H_{19}) group into position 5 of the imidazo[1,5-*a*]quinoxaline system (compound **3k**) resulted in a decrease in the bacteriostatic activity by 80 and an increase in the fungistatic activity by 20 as compared with the compound **3m**. In this connection, it can be assumed that the introduction of the hexyl (C_6H_{13}) group in the position 5 of the imidazo[1,5-*a*]quinoxaline system leads to the improvement of the bacteriostatic properties of imidazo[1,5-*a*]quinoxaline derivatives, and its presence in the position 1 of the pyridine ring contributes to the fungistatic properties.

Compounds **4a–d**, **6** consist of two *N*-alkyl-pyridine moieties (Fig. 3). The data in Table 3 show that these substances are inferior to the antimicrobial activity than the compounds listed in Table 2. Compounds **4a** and **4b** contain connecting bridges at positions 1, with the nitrogen atoms of the pyridine rings of each of the fragments. As can be seen from the data in Table 3 both compounds are inactive against bacteria. However, if the number of the methylene groups in the connecting bridges decreases from 6 as in compound **4b** to 4 as in compound **4a**, the fungistatic activity increases against the yeast *C. albicans*. Imidazo[1,5-*a*]quinoxaline compounds **4c** and **4d** containing hexyl (C_6H_{13}) and nonyl (C_9H_{19}) groups at position 5, respectively, exhibit bacteriostatic (against Gram-positive bacteria) as well as fungistatic activity (against the yeast *C. albicans*). The cyclophane **6** examined in this group exhibits a low antimicrobial activity against Gram-positive bacteria and yeast.

Compounds with the highest bacteriostatic and fungistatic efficacy were further screened for their bactericidal and fungicidal activities (Table 4). The screened compounds exhibit bactericidal and fungicidal activities in the regions of 5–500 μg/ml and 50–500 μg/ml concentrations, respectively.

Compound **3l** ranks bellow the bactericidal and fungicidal activities of the drug Ciprofloxacin and Clotrimazole, but also reveals an inhibitory effect at 5 μg/ml against Gram-positive bacteria *S. aureus*, similar to the MBC as the antibionic Norfloxacin. Its fungicidal activity at 50 μg/ml against yeast *C. albicans* and at 250 μg/ml against the *T. mentagrophytes*, is the same MBC as the antifungal drug Amphotericin B.

Table 2
Antibacterial and antifungal activity of imidazo[1,5-*a*]quinoxaline derivatives **3** *in vitro*.

Entry	Compound	R^1	R^2	R^3	X^-	Minimal inhibitory concentration (MICs) μg/mL						
						Sa	Bc	Ec	Pa	An	Tm	Ca
1	**2a**·HCl	H	Ph	–		>500	>500	>500	>500	>500	>500	>500
2	**2h**·HCl	H	Ph	–		>500	>500	>500	>500	>500	>500	>500
3	**3a**	H	Ph	Bn	Cl	31.3	125	>500	>500	>500	500	62.5
4	**3b**	CH_3	Ph	Bn	Cl	31.3	125	>500	>500	>500	500	62.5
5	**3c**	C_3H_7	Ph	Bn	Cl	62.5	125	>500	>500	>500	>500	>500
6	**3d**	C_6H_{13}	Ph	Bn	Cl	0.97	6.3	>500	>500	>500	500	7.8
7	**3e**	C_9H_{19}	Ph	Bn	Cl	1.95	12.5	>500	>500	>500	500	3.9
8	**3f**	$C_{18}H_{37}$	Ph	Bn	Cl	500	>500	>500	>500	>500	>500	>500
9	**3g**	H	CH_3	Bn	Cl	500	>500	>500	>500	>500	>500	>500
10	**3i**	H	Ph	C_6H_{13}	Br	62.5	125	>500	>500	>500	>500	250
11	**3l**	C_6H_{13}	Ph	C_9H_{19}	Br	0.97	0.97	500	>500	>500	250	0.97
12	**3n**	C_9H_{19}	Ph	C_9H_{19}	Br	1.5	6.3	>500	>500	>500	500	50
13	**3h**	H	Ph	C_4H_9	I	500	>500	>500	>500	>500	>500	>500
14	**3j**	C_6H_{13}	Ph	C_6H_{13}	I	62.5	62.5	250	500	>500	>500	15.6
15	**3k**	C_9H_{19}	Ph	C_6H_{13}	I	62.5	125	250	>500	>500	>500	25
16	**3m**	C_6H_{13}	Ph	C_9H_{19}	I	0.78	0.78	>500	>500	>500	>500	500
17	**3o**	C_9H_{19}	Ph	C_9H_{19}	I	7.8	7.8	>500	>500	>500	>500	>500
18	Ciprofloxacin					0.25	0.25	0.5	0.5			
19	Ofloxacin					0.97	1.5	0.5	3.1			
20	Norfloxacin					2.42	7.8	1.5	3.0			
21	Clotrimazole										3.13	0.39
22	Amphotericin B									20	3.13	0.97

The tests were performed in duplicate and repeated twice; *Pa*, *Pseudomonas aeruginosa*; *Ec*, *Escherichia coli*; *Sa*, *Staphylococcus aureus*; *Bc*, *Bacillus cereus*; *An*, *Aspergillus niger*; *Tm*, *Trichophyton mentagrophytes*; *Ca*, *Candida albicans*.

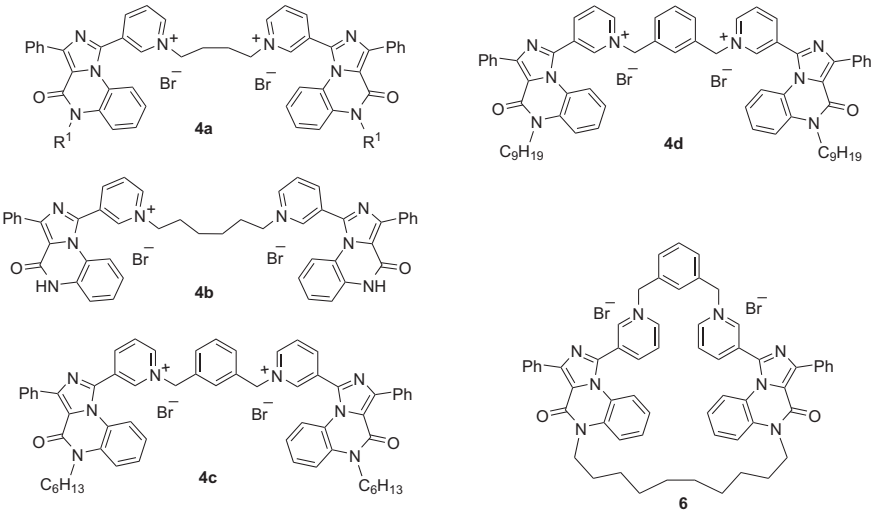


Fig. 3. Imidazo[1,5-*a*]quinoxalines with the two *N*-alkylpyridine fragments.

Table 3
Antibacterial and antifungal activity of imidazo[1,5-*a*]quinoxaline derivatives **4** with two *N*-alkylpyridine fragments *in vitro*.

Entry	Compound	Minimal inhibitory concentration (MICs) μg/ml						
		Sa	Bc	Ec	Pa	An	Tm	Ca
1	**4a**	>500	>500	>500	>500	>500	250	3.9
2	**4b**	500	>500	>500	>500	>500	>500	>500
3	**4c**	12.5	62.5	>500	>500	>500	>500	15.6
4	**4d**	3.9	15.6	500	>500	>500	500	7.8
5	**6**	125	>500	>500	>500	>500	>500	250

The tests were performed in duplicate and repeated twice; *Pa*, *Pseudomonas aeruginosa*; *Ec*, *Escherichia coli*; *Sa*, *Staphylococcus aureus*; *Bc*, *Bacillus cereus*; *An*, *Aspergillus niger*; *Tm*, *Trichophyton mentagrophytes*; *Ca*, *Candida albicans*.

Toxicity of more effective (imidazo[1,5-*a*]quinoxalin-4-on-1-yl)-1-pyridinium halides was determined in mice (Table 5).

It should be pointed out that the levels of acute toxicity for Gramicidin S by the intraperitoneal introductions is 26–30 mg/kg, while for Amphotericin B is 27.74 mg/kg. Thus according to the levels of acute toxicity for mammals the (imidazo[1,5-*a*]quinoxalin-4-on-1-yl)-1-pyridinium halides can be considered as moderately toxic compounds.

4. Conclusions

The antibacterial and antifungal activity of a series of imidazo [1,5-*a*]quinoxaline derivatives has been described. The data obtained have disclosed the dependence of the antimicrobial activity on the number of *N*-alkyl-pyridine moieties and on the nature of the substituents both in the position 1 of a pyridine ring and in the position 5 of an imidazo[1,5-*a*]quinoxaline system. It has been established that the compounds with one *N*-alkyl-pyridine fragment are most active. The introduction of the alkyl chain (C_6H_{13}) in the position 1 of the pyridine ring increases the fungistatic activity of imidazo[1,5-*a*]quinoxaline derivatives, and its presence in the 5 position of the imidazo[1,5-*a*]quinoxaline system tends to improve the bacteriostatic properties. Imidazo[1,5-*a*]quinoxaline, with bromine as a counter-ion, with the substituent (C_9H_{19}) in position 1 of the pyridine ring and the substituent (C_6H_{13}) in position 5 of the imidazoquinoxaline system (**3l**) is the best for bacteriostatic, bactericidal, fungistatic, and fungicidal properties. In this case, the antibacterial and antifungal activities of this compound is comparable to the antimicrobial activity of the known drugs – Ofloxacin, Norfloxacin and Amphotericin B.

Table 4
Bactericidal and fungicidal activity of imidazo[1,5-*a*]quinoxaline derivatives *in vitro*.

Compound	MBC μg/ml	MFC μg/mL					
	Sa	Bc	Ec	Pa	An	Tm	Ca
3d	50	>500	>500	>500	>500	>500	500
3e	50	>500	>500	>500	>500	>500	500
3l	5	50	500	>500	>500	250	50
3m	5	500	>500	>500	>500	>500	500
3n	50	500	>500	>500	>500	>500	500
3o	500	>500	>500	>500	>500	>500	>500
Ciprofloxacin	0.25	0.25	0.5	0.5			
Norfloxacin	5	12.5	5	5			
Clotrimazole						3.13	0.39
Amphotericin B					250	250	50

MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration. The tests were performed in duplicate and repeated twice; *Pa*, *Pseudomonas aeruginosa*; *Ec*, *Escherichia coli*; *Sa*, *Staphylococcus aureus*; *Bc*, *Bacillus cereus*; *An*, *Aspergillus niger*; *Tm*, *Trichophyton mentagrophytes*; *Ca*, *Candida albicans*.

5. Experimental

5.1. Chemistry

5.1.1. Materials

The melting points were determined on a Boetius hot-stage apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Bruker Vector-22 spectrometer. NMR experiments were carried out with Bruker spectrometers AVANCE-400 (400.1 MHz (^{1}H), 100.6 MHz (^{13}C)) and AVANCE-600 (600.1 MHz (^{1}H), 150.9 MHz (^{13}C)). Chemical shifts were reported on the δ (ppm) scale and are relative to the residual ^{1}H and ^{13}H signal of DMSO-d_6 and $CDCl_3$. The MALDI mass spectra were obtained on a Bruker UltraFlex III MALDI TOF/TOF instrument with *p*-nitroaniline as a matrix. The elemental analyses were carried out at the microanalysis laboratory of the Arbuzov Institute of Organic and Physical Chemistry, Russian Academy of Sciences. The 3-imidazo[1,5-*a*] quinoxalin-4-ones **2a**, **b**, **g**, **h** were synthesized according to the reported methods [25].

5.1.2. General procedure for the synthesis of imidazo[1,5-a] quinoxaline (**2c**–**f**)

A suspension of 3-benzoylquinoxaline-2(1*H*)-one (**1a**) (1.0 g, 4.0 mmol), KOH (0.50 g, 8.9 mmol), appropriate alkylbromide (4.8 mmol), and dioxane (50 mL) was refluxed for 12 h. After cooling to room temperature, the reaction mixture was poured into water (150 mL). The mixture was washed with CH_2Cl_2 (3× 40 mL),

Table 5
Toxicity of (imidazo[1,5-*a*]quinoxalin-4-on-1-yl)-1-pyridinium halides **3a**, **3d**, **3h**, **3i**, **3l**, **3m** and **4d**.

Compound	LD50, mice, mg/kg
3a	65.7 (47.3÷79.7)
3d	61.1 (47.7÷78.5)
3h	63.2 (52.1÷75.3)
3i	75.8 (65.2÷87.1)
3l	120.5 (39.3÷69.2)
3m	52.5 (36.4÷65.7)
4d	108.2 (86.7÷140.2)

and the combined organic phases were then washed with water (3× 10 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. Hexane (15 mL) was added to the residue. The reaction mixture was left overnight. The solution was separated from the residue. The crude product **2c**–**f** was dried. A solution of 3-picolylamine (4.2 mmol) in DMSO (10 mL) was added to the residue. The reaction mixture was stirred for 8 h at 150 °C, and left overnight at room temperature, wherein the crystals of the product were precipitated out. The crystals were collected by suction filtration, washed with EtOH (10 mL) and dried. The mother solution was poured into water (20 mL), the product extracted with methylene chloride (3× 7 mL). The combined methylene chloride solutions were washed with water (3× 3 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The crude product was recrystallized from acetonitrile and the additional amount of compound **2** was obtained.

*5.1.2.1. 3-Phenyl-5-propyl-1-(pyridin-3-yl)imidazo[1,5-a]quinoxalin-4-one (**2c**).* Yield 51%. White powder, mp 182–184 °C (CH_3CN). IR (ν_{max}, cm^{-1}, KBr): 3105, 3071, 2958, 2929, 2871, 1658, 1611, 1595, 1502, 1484, 1467, 1416, 1393, 1301, 1242, 1114, 1025, 748, 727, 697, 670. ^1H NMR (400 MHz, $CDCl_3$) δ_H: 1.07 (t, 3H, J = 7.4 Hz, CH_3), 1.75–1.90 (m, 2H, CH_2), 4.21 (t, 2H, J = 7.9 Hz, NCH_2), 6.94 (ddd, 1H, J = 8.5, 6.7, 1.9 Hz, H8-Q), 7.28–7.49 (m, 6H, ArH), 7.50 (ddd, 1H, J = 7.9, 4.9, 0.8 Hz, H5-Py), 8.05 (ddd, 1H, J = 7.9, 2.2, 1.7 Hz, H4-Py), 8.10–8.15 (m, 2H, *o*-Ph), 8.82 (dd, 1H, J = 4.9, 1.7 Hz, H6-Py), 8.98 (dd, 1H, J = 2.2, 0.8 Hz, H2-Py). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C: 11.50, 20.77, 43.58, 116.21, 118.08, 118.84, 122.44, 122.83, 123.90, 127.55, 128.11, 128.81, 128.83, 130.15, 130.73, 132.91, 137.36, 141.52, 146.23, 150.41, 151.10, 155.48. MS (MALDI TOF) = 381 $[MH]^+$, 403 $[M + Na]^+$, 419 $[M + K]^+$. Found: C, 75.90; H, 5.23; N, 14.62. $C_{24}H_{20}N_4O$ requires: C, 75.77; H, 5.30; N,14.73%.

*5.1.2.2. 5-Hexyl-3-phenyl-1-(pyridin-3-yl)imidazo[1,5-a]quinoxalin-4-one (**2d**).* Yield 57%. White powder, mp 164–166 °C (CH_3CN). IR (ν_{max}, cm^{-1}, KBr): 3104, 3053, 2952, 2922, 2863, 2846, 1655, 1610, 1592, 1567, 1484, 1461, 1447, 1412, 1396, 1355, 1325, 1300, 1249, 1198, 1182, 1118, 1052, 1025, 751, 728, 715, 697, 670. ^1H NMR (400 MHz, $CDCl_3$) δ_H: 0.91 (t, 3H, J = 6.8 Hz, CH_3), 1.30–1.40 (m, 4H, CH_2), 1.43–1.53 (m, 2H, CH_2), 1.74–1.84 (m, 2H, CH_2), 4.23 (t, 2H, J = 7.8 Hz, NCH_2), 6.94 (ddd, 1H, J = 8.3, 6.8, 1.4 Hz, H8-Q), 7.30 (d, 1H, J = 7.9 Hz, H9-Q), 7.32–7.42 (m, 3H, H6,7-Q, *p*-Ph), 7.47 (dd, 2H, J = 7.6, 7.6 Hz, *m*-Ph), 7.50 (dd, 1H, J = 8.1, 5.0 Hz, H5-Py), 8.06 (ddd, 1H, J = 8.1, 1.6, 1.6 Hz, H4-Py), 8.13 (dd, 2H, J = 7.9, 1.3 Hz, *o*-Ph), 8.82 (br dd, 1H, J = 5.0, 1.6 Hz, H6-Py), 8.97 (br d, 1H, J = 1.6 Hz, H2-Py). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C: 14.16, 22.76, 26.88, 27.40, 31.70, 42.11, 116.15, 118.04, 118.83, 122.39, 122.78, 123.89, 127.54, 128.07, 128.09, 128.77, 130.11, 130.67, 132.87, 137.39, 141.42, 146.13, 150.30, 150.99, 155.37. MS (MALDI TOF) = 423 $[MH]^+$. Found: C, 76.87; H, 6.29; N, 13.12. $C_{27}H_{26}N_4O$ requires: C, 76.75; H, 6.20; N,13.26%.

*5.1.2.3. 5-Nonyl-3-phenyl-1-(pyridin-3-yl)imidazo[1,5-a]quinoxalin-4-one (**2e**).* Yield 51%. White powder, mp 148–150 °C (CH_3CN). IR (ν_{max}, cm^{-1}, KBr): 3102, 3053, 2921, 2848, 1655, 1610, 1592, 1567, 1501, 1485, 1469, 1412, 1396, 1355, 1326, 1300, 1280, 1250, 1183, 1119, 1025, 1025, 813, 783, 751, 727, 715, 697, 670. ^1H NMR (400 MHz, $CDCl_3$) δ_H: 0.88 (t, 3H, J = 6.8 Hz, CH_3), 1.20–1.32 (m, 8H, CH_2), 1.33–1.42 (m, 2H, CH_2), 1.42–1.52 (m, 2H, CH_2), 1.72–1.86 (m, 2H, CH_2), 4.23 (t, 2H, J = 7.8 Hz, NCH_2), 6.94 (ddd, 1H, J 8.4, 6.8, 1.6, H8-Q), 7.30 (d, 1H, J = 8.6 Hz, H9-Q), 7.32–7.43 (m, 3H, H6,7-Q, *p*-Ph), 7.47 (dd, 2H, J = 7.6, 7.3 Hz, *m*-Ph), 7.52 (dd, 1H, J = 7.9, 5.0 Hz, H5-Py), 8.07 (d, 1H, J = 7.9 Hz, H4-Py), 8.13 (d, 2H, J = 7.3 Hz, *o*-Ph), 8.82 (br d, 1H, J = 5.0 Hz, H6-Py), 8.97 (br s, 1H, H2-Py). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C: 14.25, 22.82, 27.24, 27.46, 29.40, 29.55, 29.71, 32.01, 42.15, 116.22, 118.06, 118.89, 122.43, 122.78, 124.01, 127.60, 128.11, 128.82, 128.92, 130.13, 130.71, 132.86, 137.63, 141.31, 146.20, 150.09, 150.76, 155.39. MS (MALDI TOF) = 465 $[MH]^+$. Found: C, 77.47; H, 6.79; N, 12.15. $C_{30}H_{32}N_4O$ requires: C, 77.56; H, 6.94; N,12.06%.

*5.1.2.4. 5-Octadecyl-3-phenyl-1-(pyridin-3-yl)imidazo[1,5-a]quinoxalin-4-one (**2f**).* Yield 44%. White powder, mp 134–137 °C (CH_3CN). IR (ν_{max}, cm^{-1}, KBr): 2956, 2848, 1655, 1610, 1593, 1501, 1484, 1467, 1412, 1396, 1326, 1300, 1252, 1182, 1119, 1025, 751, 716, 697. ^1H NMR (400 MHz, $CDCl_3$) δ_H: 0.88 (t, 3H, J = 6.8 Hz, CH_3), 1.20–1.32 (m, 26H, CH_2), 1.33–1.42 (m, 2H, CH_2), 1.42–1.52 (m, 2H, CH_2), 1.74–1.84 (m, 2H, CH_2), 4.23 (t, 2H, J = 7.8 Hz, NCH_2), 6.99 (d, 1H, J = 8.3, 7.0, 1.4 Hz, H8-Q), 7.25–7.44 (m, 4H, H6,7,9-Q, *p*-Ph), 7.47 (dd, 2H, J = 7.6, 7.1 Hz, *m*-Ph), 7.69 (dd, 1H, J = 7.9, 5.2, H5-Py), 8.13 (dd, 2H, J = 7.9, 1.4, *o*-Ph), 8.30 (ddd, 1H, J = 7.9, 1.6, 1.5 Hz, H4-Py), 8.85 (dd, 1H, J = 5.2, 1.5 Hz, H6-Py), 9.02 (br d, 1H, J = 1.6 Hz, H2-Py). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C: 14.27, 22.85, 27.25, 27.46, 29.53, 29.56, 29.70–29.55 (10C), 32.10, 42.15, 116.18, 118.06, 118.85, 122.39, 122.81, 123.90, 127.55, 128.10, 128.78, 129.79, 130.13, 130.70, 132.89, 137.39, 141.45, 146.17, 150.34, 151.02, 155.39. MS (MALDI TOF) = 591 $[MH]^+$. Found: C, 79.43; H, 8.69; N, 9.55. $C_{39}H_{50}N_4O$ requires: C, 79.28; H, 8.53; N, 9.48%.

*5.1.3. General procedure for the synthesis of imidazo[1,5-a] quinoxaline derivatives with the pyridinium salt fragments (**3**, **4**)*

A mixture of imidazo[1,5-*a*]quinoxaline **2** (0.40 mmol) and alkylhalide R^3X (0.60 mmol) or dibromoalkane $BrCH_2ZCH_2Br$ (0.19 mmol) *a*. in DMF (2.5 mL) was stirred for 16 h at 125 °C (for synthesizing **3a**, **g**–**i** and **4a**, **b**) *b*. in acetonitrile (10 mL) was refluxed for 24 h (for synthesizing **3b**–**f**, **j**–**o** and **4c**, **d**).

When synthesizing **3a**, **3g** the reaction mixture was left overnight at room temperature, while the crystals of the product were precipitated, collected by suction filtration, washed with CH_3CN (5 mL) and solvent was evaporated and ether (20 mL) was added to the residue. The resulting precipitate was filtered off and washed with Et_2O (2 × 10 mL). When synthesizing **3h**, **i** after cooling to room temperature and standing overnight, Et_2O (20 mL) was poured into the reaction mixture. After collection by filtration the product was washed with Et_2O (20 mL) and dried in vacuum. When synthesizing **3b**–**f**, **j**–**o** the solvent was removed in vacuum and the residue was purified by flash column chromatography on Silicagel (EtOH:CH_2Cl_2 = 1:30 → 1:10) (Table 1). When synthesizing **4a**, **b**, **4c**, **d** the reaction mixture was left overnight at room temperature, while the crystals of the product were precipitated, collected by suction filtration, washed with CH_3CN (5 mL) and dried in vacuum.

*5.1.3.1. 1-Benzyl-3-(3-phenylimidazo[1,5-a]quinoxalin-4(5H)-on-1-yl)pyridinium chloride (**3a**).* Yield 87%. White powder, mp 333–335 °C (dec.). IR (ν_{max}, cm^{-1}, Nujol mull): 3220–2500, 1666, 1618, 1508, 1484, 1396, 1329, 1301, 1274, 1248, 1157, 1075, 1032, 851, 815, 739, 698, 523. ^1H NMR (400 MHz, DMSO-d_6) δ_H: 5.99 (s, 2H, $\underline{CH_2}$Ph), 6.92 (dd, 1H, J = 8.5, 7.5 Hz, H8-Q), 7.20 (d, 1H, J = 8.5 Hz, H9-Q), 7.36–7.53 (m, 8H, ArH), 7.60–7.66 (m, 2H, $CH_2\underline{Ph}$-o), 8.19 (d, 2H,

J = 7.5 Hz, C3-Ph-*o*), 8.42 (dd, 1H, J = 7.8, 6.2 Hz, H5-Py), 9.02 (d, 1H, J = 7.8 Hz, H4-Py), 9.49 (d, 1H, J = 6.2 Hz, H6-Py), 9.79 (s, 1H, H2-Py), 11.70 (s, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ_C: 63.66, 116.94, 117.48, 119.91, 120.63, 122.30, 127.62, 127.73, 128.42, 128.73, 129.05, 129.16, 129.39, 129.46, 129.71, 131.92, 132.25, 133.83, 137.69, 144.17, 145.24, 145.85, 145.92, 154.83. MS (MALDI TOF) = 429 $[M - Cl^-]^+$. Found: C, 72.18; H, 4.39; N, 12.02; Cl, 7.59. $C_{28}H_{21}N_4OCl$ requires: C, 72.33; H, 4.55; N,12.05; Cl, 7.63%.

*5.1.3.2. 1-Benzyl-3-(5-methyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)pyridinium chloride (**3b**).* Yield 71%. White powder, mp > 205 °C (dec.). IR (ν_{max}, cm^{-1}, KBr): 3022, 2990, 1647, 1612, 1590, 1522, 1487, 1447, 1400, 1368, 1330, 1300, 1260, 1162, 1105, 980, 756, 705, 671. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 3.55 (s, 3H, CH_3), 6.35 (s, 2H, $\underline{CH_2}$Ph), 7.05 (ddd, 1H, J = 8.6, 7.3, 1.4 Hz, H8-Q), 7.32 (dd, 1H, J = 8.6, 1.4 Hz, H9-Q), 7.30–7.45 (m, 7H, ArH), 7.63 (dd, 1H, J = 8.3, 1.6 Hz, H6-Q), 7.66–7.70 (m, 2H, ArH), 7.97–8.02 (m, 2H, C3-Ph-o), 8.20 (dd, 1H, J = 8.2, 6.0 Hz, H5-Py), 8.58 (d, 1H, J = 8.2 Hz, H4-Py), 9.33 (s, 1H, H2-Py), 10.08 (d, 1H, J = 6.0 Hz, H6-Py). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ_C: 28.88, 63.55, 116.72, 117.88, 119.36, 121.30, 122.70, 127.66, 127.90, 128.48, 128.80, 129.05, 129.15, 129.39, 129.59, 130.81, 131.76, 132.22, 133.81, 137.46, 144.51, 145.15, 145.67, 145.76, 154.79. MS (MALDI TOF) = 443 $[M - Cl^-]^+$. Found: C, 72.58; H, 4.69; N, 11.82; Cl, 7.38. $C_{29}H_{23}N_4OCl$ requires: C, 72.72; H, 4.84; N,11.70; Cl, 7.40%.

*5.1.3.3. 1-Benzyl-3-(5-propyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)pyridinium chloride (**3c**).* Yield 70%. White powder, mp 193–196 °C (dec.). IR (ν_{max}, cm^{-1}, KBr): 3079, 3037, 2960, 2932, 2874, 1648, 1608, 1589, 1555, 1520, 1486, 1450, 1401, 1324, 1299, 1258, 1185, 1165, 1118, 1058, 785, 752, 706, 674. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.06 (t, 3H, J = 7.4 Hz, CH_3), 1.75–1.85 (m, 2H, CH_2), 4.15 (t, 2H, J = 7.9 Hz, NCH_2), 6.36 (s, 2H, $\underline{CH_2}$Ph), 7.11 (dd, 1H, J = 8.2, 7.4 Hz, H8-Q), 7.33 (d, 1H, J = 8.2 Hz, H9-Q), 7.36–7.47 (m, 7H, ArH), 7.49 (d, 1H, J = 8.0 Hz, H6-Q), 7.62–7.70 (m, 2H, ArH), 8.04 (d, 2H, J = 7.0 Hz, C3-Ph-o), 8.21 (dd, 1H, J = 7.7, 6.1, H5-Py), 8.59 (d, 1H, J = 7.7 Hz, H4-Py), 9.20 (s, 1H, H2-Py), 10.04 (br d, 1H, J = 6.1 Hz, H6-Py). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ_C: 11.04, 20.18, 42.54, 63.74, 116.70, 118.21, 119.20, 121.42, 122.59, 127.69, 127.98, 128.48, 128.76, 129.00, 129.19, 129.41, 129.62, 129.79, 131.93, 132.28, 133.80, 137.37, 144.52, 145.20, 145.72, 145.83, 154.34. MS (MALDI TOF) = 471 $[M - Cl^-]^+$. Found: C, 73.56; H, 5.49; N, 11.07; Cl, 6.88. $C_{31}H_{27}N_4OCl$ requires: C, 73.44; H, 5.37; N,11.05; Cl, 6.99%.

*5.1.3.4. 1-Benzyl-3-(5-hexyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)pyridinium chloride (**3d**).* Yield 79%. White powder, mp 232–234 °C (dec.). IR (ν_{max}, cm^{-1}, KBr): 3058, 2988, 2956, 2854, 1666, 1611, 1591, 1486, 1457, 1448, 1402, 1376, 1323, 1299, 1253, 1176, 1154, 1114, 755, 744, 692. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.90 (t, 3H, J = 7.0 Hz, CH_3), 1.28–1.38 (m, 4H, CH_2), 1.40–1.50 (m, 2H, CH_2), 1.70–1.80 (m, 2H, CH_2), 4.15 (t, 2H, J = 7.9 Hz, NCH_2), 6.38 (s, 2H, $\underline{CH_2}$Ph), 7.09 (dd, 1H, J = 8.2, 7.4 Hz, H8-Q), 7.33 (д, 1H, J = 8.2 Hz, H9-Q), 7.35–7.48 (m, 8H, ArH), 7.65–7.72 (m, 2H, ArH), 8.03 (dd, 2H, J = 8.2, 1.5 Hz, C3-Ph-o), 8.21 (dd, 1H, J = 7.9, 6.0 Hz, H5-Py), 8.58 (d, 1H, J = 7.9 Hz, H4-Py), 9.24 (s, 1H, H2-Py), 10.13 (br d, 1H, J = 6.0, H6-Py). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C: 14.15, 22.76, 26.88, 27.39, 31.66, 42.43, 65.59, 116.34, 118.78, 120.17, 121.44, 123.89, 128.18, 128.58, 128.73, 129.29, 129.98, 130.07, 130.32, 130.40, 130.47, 131.95, 132.33, 132.44, 136.46, 144.19, 144.69, 146.67, 146.84, 154.81. MS (MALDI TOF) = 513 $[M - Cl^-]^+$. Found: C, 74.46; H, 6.19; N, 10.17; Cl, 6.29. $C_{34}H_{33}N_4OCl$ requires: C, 74.37; H, 6.06; N,10.20; Cl, 6.46%.

*5.1.3.5. 1-Benzyl-3-(5-nonyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)pyridinium chloride (**3e**).* Yield 69%. White powder, mp 182–184 °C. IR (ν_{max}, cm^{-1}, KBr): 2929, 2848, 2723, 1669, 1613, 1591, 1485, 1458, 1402, 1376, 1300, 1256, 1216, 1155, 1116, 755, 746, 702, 692, 667. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.87 (t, 3H, J = 6.8 Hz, CH_3), 1.22–1.30 (m, 8H, CH_2), 1.30–1.40 (m, 2H, CH_2), 1.40–1.50 (m, 2H, CH_2), 1.70–1.80 (m, 2H, CH_2), 4.15 (t, 2H, J = 7.8 Hz, NCH_2), 6.38 (s, 2H, $\underline{CH_2}$Ph), 7.10 (dd, 1H, J 8.6, 7.6 Hz, H8-Q), 7.32 (d, 1H, J = 8.6 Hz, H9-Q), 7.40–7.70 (m, 10H, ArH), 8.03 (dd, 2H, J = 8.1, 1.4 Hz, C3-Ph-o), 8.23 (dd, 1H, J 7.9, 5.6 Hz, H5-Py), 8.60 (d, 1H, J = 7.9, Hz, H4-Py), 9.27 (br s, 1H, H2-Py), 10.06 (br d, 1H, J = 5.6 Hz, H6-Py). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C: 14.22, 22.78, 27.20, 27.42, 29.35, 29.48, 29.67, 31.97, 42.29, 61.31, 116.43, 118.78, 120.20, 121.43, 123.82, 128.16, 128.55, 128.66, 129.22, 130.03, 130.26, 130.38, 130.83, 131.24, 132.02, 132.30, 132.51, 136.48, 144.17, 144.70, 146.69, 146.80, 154.82. MS (MALDI TOF) = 555 $[M - Cl^-]^+$. Found: C, 75.06; H, 6.59; N, 9.57; Cl, 5.87. $C_{37}H_{39}N_4OCl$ requires: C, 75.17; H, 6.65; N,9.48; Cl, 6.00%.

*5.1.3.6. 1-Benzyl-3-(5-octadecyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)pyridinium chloride (**3f**).* Yield 74%. White powder, mp 225–227 °C (dec.). IR (ν_{max}, cm^{-1}, KBr): 3059, 2989, 2919, 2850, 1667, 1611, 1591, 1486, 1468, 1458, 1403, 1376, 1300, 1253, 1179, 1115, 756, 744, 702, 692. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.88 (t, 3H, J = 6.6 Hz, CH_3), 1.22–1.30 (m, 26H, CH_2), 1.30–1.40 (m, 2H, CH_2), 1.40–1.50 (m, 2H, CH_2), 1.70–1.82 (m, 2H, CH_2), 4.19 (t, 2H, J = 7.5 Hz, NCH_2), 6.30 (s, 2H, $\underline{CH_2}$Ph), 7.13–7.22 (m, 1H, H8-Q), 7.34 (d, 1H, J = 8.4 Hz, H9-Q), 7.40–7.50 (m, 7H, ArH), 7.65 (d, 1H, J = 7.7 Hz, H6-Q), 7.65–7.75 (m, 2H, ArH), 8.07 (d, 2H, J = 7.3, C3-Ph-o), 8.17 (dd, 1H, J = 8.0, 5.6 Hz, H5-Py), 8.60 (d, 1H, J = 8.0 Hz, H4-Py), 9.34 (br s, 1H, H2-Py), 9.81 (br s, 1H, H6-Py). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C: 14.29, 22.87, 27.26, 27.47, 29.53, 29.54, 29.70–29.55 (10C), 32.11, 42.47, 65.94, 116.52, 118.97, 120.33, 121.36, 124.00, 128.27, 128.62, 128.75, 129.40, 129.69, 130.08, 130.11, 130.39, 130.54, 130.59, 130.60, 131.94, 136.24, 136.27, 144.15, 144.63, 146.75, 154.79. MS (MALDI TOF) = 681 $[M - Cl^-]^+$. Found: C, 77.12; H, 8.05; N, 7.87; Cl, 4.84. $C_{46}H_{57}N_4OCl$ requires: C, 77.01; H, 8.01; N,7.81; Cl, 4.94%.

*5.1.3.7. 1-Benzyl-3-(3-methylimidazo[1,5-a]quinoxalin-4(5H)-on-1-yl)pyridinium chloride (**3g**).* Yield 64%. White powder, mp 326–328 °C (dec.). IR (ν_{max}, cm^{-1}, Nujol mull): 3220–2500, 1669, 1617, 1571, 1519, 1503, 1412, 1325, 1269, 1246, 1203, 1161, 1140, 1098, 1030, 930, 843, 820, 746, 736, 709, 682, 629, 584. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.66 (s, 3H, Me), 5.97 (s, 2H, $\underline{CH_2}$Ph), 6.89 (dd, 1H, J = 8.4, 7.9 Hz, H8-Q), 7.19 (d, 1H, J = 8.4 Hz, H9-Q), 7.30–7.43 (m, 2H, H6,7-Q), 7.47–7.55 (m, 3H, *m,p*-Ph), 7.60–7.66 (m, 2H, *o*-Ph), 8.38 (ddd, 1H, J = 8.2, 6.2, 1.7 Hz, H5-Py), 8.93 (d, 1H, J = 8.2 Hz, H4-Py), 9.45 (d, 1H, J = 6.2 Hz, H6-Py), 9.73 (s, 1H, H2-Py), 11.55 (s, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ_C: 13.93, 63.64, 117.10, 117.11, 120.23, 120.87, 122.22, 125.40, 128.61, 128.96, 129.17, 129.39, 129.87, 132.04, 133.85, 136.47, 142.56, 145.10, 145.54, 145.73, 155.53. MS (MALDI TOF) = 367 $[M - Cl^-]^+$. Found: C, 68.52; H, 4.66; N, 13.87; Cl, 8.85. $C_{23}H_{19}N_4OCl$ requires: C, 68.57; H, 4.75; N,13.91; Cl, 8.80%.

*5.1.3.8. 1-Butyl-3-(3-phenylimidazo[1,5-a]quinoxalin-4(5H)-on-1-yl) pyridinium iodide (**3h**).* Yield 95%. Light-yellow powder, mp > 215 °C (dec.). IR (ν_{max}, cm^{-1}, Nujol mull): 3220–2500, 1667, 1616, 1580, 1547, 1522, 1484, 1421, 1395, 1329, 1302, 1275, 1247, 1202, 1159, 786, 766, 753, 722, 698, 682, 673. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 0.96 (t, 3H, J = 7.2 Hz, CH_3), 1.35–1.44 (m, 2H, $CH_3\underline{CH_2}$), 1.97–2.04 (m, 2H, $NCH_2\underline{CH_2}$), 4.70 (t, 2H, J = 7.2 Hz, $N\underline{CH_2}CH_2$), 6.99–7.04 (m, 1H, H8-Q), 7.28 (d, 1H, J = 8.5 Hz, H9-Q), 7.40–7.46 (m, 3H, H6,7-Q, *p*-Ph), 7.47–7.52 (m, 2H, *m*-Ph), 8.21 (dd, 2H, J = 7.5, 1.3 Hz, *o*-Ph), 8.39 (dd, 1H, J = 8.1, 6.3 Hz, H5-Py), 8.99 (d, 1H, J 8.1 Hz, H4-Py), 9.33 (d, 1H, J = 6.3 Hz, H6-Py), 9.66 (s, 1H, H2-Py), 11.66 (s, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ_C: 13.32, 18.78, 32.49, 61.10, 116.90, 117.68, 119.91, 120.69, 122.32, 127.73, 127.74, 128.33, 128.45, 129.47, 129.73, 131.62, 132.27, 137.82, 144.19,

145.11, 145.37, 145.76, 154.90. MS (MALDI TOF) = 395 [M − I$^-$]$^+$. Found: C, 57.52; H, 4.48; N, 10.77; I, 24.19. $C_{25}H_{23}N_4OI$ requires: C, 57.48; H, 4.44; N, 10.73; I, 24.29%.

*5.1.3.9. 1-Hexyl-3-(3-phenylimidazo[1,5-a]quinoxalin-4(5H)-on-1-yl)pyridinium bromide (**3i**).* Yield 87%. White powder, mp 173–175 °C (dec.). IR (ν_{max}, cm^{-1}, Nujol mull): 3220–2500, 1671, 1616, 1556, 1524, 1504, 1486, 1427, 1400, 1326, 1304, 1275, 1247, 1162, 1009, 818, 769, 746, 698, 688, 675, 526. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 0.89 (t, 3H, J = 7.0 Hz, CH_3), 1.29–1.41 (m, 6H, $CH_3\underline{CH_2}$), 1.96–2.05 (m, 2H, $NCH_2\underline{CH_2}$), 4.70 (t, 2H, J = 7.4 Hz, NCH_2), 6.97–7.03 (m, 1H, H8-Q), 7.28 (d, 1H, J = 8.6 Hz, H9-Q), 7.40–7.45 (m, 3H, H6,7-Q, *p*-Ph), 7.47–7.52 (m, 2H, *m*-Ph), 8.20–8.23 (m, 2H, Hz, *o*-Ph), 8.39 (dd, 1H, J = 8.1, 6.1 Hz, H5-Py), 8.99 (d, 1H, J = 8.1 Hz, H4-Py), 9.34 (уш. d, 1H, J = 6.1 Hz, H6-Py), 9.66 (s, 1H, H2-Py), 11.66 (s, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ_C: 13.75, 21.76, 25.03, 30.53, 34.21, 61.26, 116.92, 117.66, 119.90, 120.70, 122.30, 127.74, 127.75, 128.32, 128.45, 129.47, 129.75, 131.59, 132.28, 137.83, 144.17, 145.12, 145.36, 145.80, 154.89. MS (MALDI TOF) = 423 [M − Br$^-$]$^+$. Found: C, 64.50; H, 5.47; N, 11.00; Br, 15.78. $C_{27}H_{27}N_4OBr$ requires: C, 64.42; H, 5.41; N,11.13; Br, 15.87%.

*5.1.3.10. 1-Hexyl-3-(5-hexyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)pyridinium iodide (**3j**).* Yield 72%. Yellow powder, mp 113–115 °C. IR (ν_{max}, cm^{-1}, KBr): 3043, 2954, 2928, 2855, 1649, 1610, 1589, 1487, 1460, 1447, 1404, 1372, 1300, 1256, 1119, 754, 695, 670. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.85–0.95 (m, 6H, CH_3), 1.30–1.40 (m, 8H, CH_2), 1.40–1.52 (m, 4H, CH_2), 1.72–1.82 (m, 2H, CH_2), 2.10–2.20 (m, 2H, CH_2), 4.16 (t, 2H, J = 7.6 Hz, NCH_2), 4.92 (t, 2H, J = 7.9 Hz, N^+CH_2), 7.22–7.30 (m, 1H, H8-Q), 7.33 (dd, 1H, J = 8.6, 1.0 Hz, H9-Q), 7.35–7.48 (m, 4H, H7-Q, *m*,*p*-Ph), 7.70 (dd, 1H, J = 8.3, 1.0 Hz, H6-Q), 8.06 (dd, 2H, J = 8.2, 1.4 Hz, *o*-Ph), 8.30 (dd, 1H, J = 8.1, 6.2 Hz, H5-Py), 8.57 (d, 1H, J = 8.1 Hz, H4-Py), 9.10 (s, 1H, H2-Py), 9.61 (d, 1H, J = 6.2 Hz, H6-Py). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C: 14.06, 14.14, 22.52, 22.74, 26.05, 26.86, 27.38, 31.24, 31.25, 31.64, 42.40, 63.54, 116.41, 119.35, 120.29, 121.35, 124.17, 128.17, 128.61, 129.08, 129.22, 130.04, 130.40, 132.11, 132.67, 136.55, 144.07, 144.29, 146.12, 146.79, 154.88. MS (MALDI TOF) = 507 [M − I$^-$]$^+$. Found: C, 62.58; H, 6.22; N, 8.86; I, 19.87. $C_{33}H_{39}N_4OI$ requires: C, 62.46; H, 6.19; N, 8.83; I, 20.00%.

*5.1.3.11. 1-Hexyl-3-(5-nonyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)pyridinium iodide (**3k**).* Yield 69%. Yellow powder, mp 97–99 °C. IR (ν_{max}, cm^{-1}, KBr): 3046, 2954, 2923, 2852, 1648, 1609, 1589, 1521, 1486, 1459, 1402, 1374, 1298, 1258, 1169, 1120, 694, 671. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.87 (t, 3H, J = 7.0 Hz, CH_3), 0.89 (t, 3H, J = 7.0 Hz, CH_3), 1.20–1.53 (m, 18H, CH_2), 1.70–1.80 (m, 2H, CH_2), 2.10–2.20 (m, 2H, CH_2), 4.16 (t, 2H, J = 7.9 Hz, NCH_2), 4.91 (t, 2H, J = 7.6 Hz, N^+CH_2), 7.22–7.30 (m, 1H, H8-Q), 7.30 (d, 1H, J = 8.2 Hz, H9-Q), 7.35–7.48 (m, 4H, H7-Q, *m*,*p*-Ph), 7.70 (d, 1H, J = 8.1 Hz, H6-Q), 8.07 (dd, 2H, J = 7.7, 1.5 Hz, *o*-Ph), 8.30 (dd, 1H, J = 7.7, 6.1 Hz, H5-Py), 8.58 (d, 1H, J = 8.2 Hz, H4-Py), 9.10 (s, 1H, H2-Py), 9.57 (br d, 1H, J = 6.1 Hz, H6-Py). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C: 14.08, 14.25, 22.55, 22.81, 26.12, 27.24, 27.47, 29.39, 29.52, 29.71, 31.27, 31.30, 32.00, 42.44, 63.80, 116.51, 119.39, 120.49, 121.35, 124.27, 128.25, 128.74, 129.04, 129.30, 130.05, 130.51, 132.12, 132.95, 136.41, 144.05, 144.25, 146.09, 147.00, 154.91. MS (MALDI TOF) = 549 [M-I$^-$]$^+$. Found: C, 63.84; H, 6.70; N, 8.32; I, 18.67. $C_{36}H_{45}N_4OI$ requires: C, 63.90; H, 6.70; N, 8.28; I, 18.75%.

*5.1.3.12. 3-(5-Hexyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)-1-nonylpyridinium bromide (**3l**).* Yield 65%. White powder, mp 99–100 °C. IR (ν_{max}, cm^{-1}, KBr): 3049, 2953, 2925, 2854, 1646, 1610, 1589, 1522, 1503, 1487, 1463, 1447, 1403, 1374, 1339, 1322, 1299, 1259, 1203, 1178, 1148, 1116, 1072, 985, 785, 755, 718, 695, 671, 579, 555. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.86 (t, 3H, J = 7.0 Hz, CH_3), 0.90 (t, 3H, J = 7.0 Hz, CH_3), 1.20–1.32 (m, 8H, CH_2), 1.32–1.42 (m, 6H, CH_2), 1.42–1.52 (m, 4H, CH_2), 1.72–1.83 (m, 2H, CH_2), 2.09–2.22 (m, 2H, CH_2), 4.18 (t, 2H, J = 7.8 Hz, NCH_2), 5.02 (t, 2H, J = 7.4 Hz, N^+CH_2), 7.22 (dd, 1H, J = 8.3, 7.0 Hz, H8-Q), 7.34 (d, 1H, J = 8.3 Hz, H9-Q), 7.36–7.48 (m, 4H, H7-Q, *m*,*p*-Ph), 7.61 (d, 1H, J = 8.1 Hz, H6-Q), 8.06 (dd, 2H, J = 8.1, 1.4 Hz, *o*-Ph), 8.31 (dd, 1H, J = 7.6, 6.0 Hz, H5-Py), 8.59 (d, 1H, J = 7.6 Hz, H4-Py), 9.11 (s, 1H, H2-Py), 9.88 (br d, 1H, J = 6.0, Hz, H6-Py). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C: 14.17, 14.23, 22.76, 22.77, 26.46, 26.89, 27.42, 29.21, 29.30, 29.47, 31.62, 31.68, 31.94, 42.40, 63.49, 116.49, 119.14, 120.38, 121.45, 124.02, 128.24, 128.72, 128.99, 129.29, 130.03, 130.52, 132.10, 132.72, 136.42, 143.96, 144.24, 146.66, 146.94, 154.90. MS (MALDI TOF) = 549 [M − Br$^-$]$^+$. Found: C, 68.60; H, 7.31; N, 8.77; Br, 12.53. $C_{36}H_{45}N_4OBr$ requires: C, 68.67; H, 7.20; N, 8.90; Br, 12.69%.

*5.1.3.13. 3-(5-Hexyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)-1-nonylpyridinium iodide (**3m**).* Yield 68%. Yellow powder, mp 100–102 °C. IR (ν_{max}, cm^{-1}, Nujol mull): 1646, 1610, 1590, 1521, 1486, 1402, 1299, 1258, 1166, 1148, 1112, 1026, 981, 754. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.87 (t, 3H, J = 7.0 Hz, CH_3), 0.90 (t, 3H, J = 7.0 Hz, CH_3), 1.20–1.32 (m, 8H, CH_2), 1.31–1.42 (m, 6H, CH_2), 1.42–1.52 (m, 4H, CH_2), 1.72–1.82 (m, 2H, CH_2), 2.10–2.22 (m, 2H, CH_2), 4.17 (t, 2H, J 7.8, NCH_2), 4.91 (t, 2H, J = 7.7 Hz, N^+CH_2), 7.28 (dd, 1H, J = 8.0, 7.3 Hz, H8-Q), 7.33 (d, 1H, J = 8.0 Hz, H9-Q), 7.36–7.48 (m, 4H, H7-Q, *m*,*p*-Ph), 7.72 (d, 1H, J = 8.4 Hz, H6-Q), 8.07 (br d, 2H, J = 7.8 Hz, *o*-Ph), 8.30 (dd, 1H, J = 8.2 6.1 Hz, H5-Py), 8.58 (d, 1H, J = 8.2, H4-Py), 9.09 (s, 1H, H2-Py), 9.58 (d, 1H, J = 6.1 Hz, H6-Py). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C: 14.14, 14.20, 22.73, 22.74, 26.40, 26.86, 27.38, 29.18, 29.28, 29.45, 31.28, 31.64, 31.91, 42.39, 63.57, 116.39, 119.39, 120.29, 121.34, 124.20, 128.16, 128.60, 129.08, 129.21, 130.03, 130.39, 132.11, 132.68, 136.55, 144.06, 144.30, 146.05, 146.78, 154.87. MS (MALDI TOF) = 549 (M − I$^-$)$^+$. Found: C, 63.85; H, 6.66; N, 8.30; I, 18.65. $C_{36}H_{45}N_4OI$ requires: C, 63.90; H, 6.70; N, 8.28; I, 18.75%.

*5.1.3.14. 1-Nonyl-3-(5-nonyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)pyridinium bromide (**3n**).* Yield 64%. White powder, mp 165–167 °C (dec.). IR (ν_{max}, cm^{-1}, KBr): 3052, 2954, 2924, 2852, 1650, 1611, 1487, 1462, 1447, 1404, 1372, 1299, 1251, 1117, 754, 695, 671. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.83–0.90 (m, 6H, CH_3), 1.20–1.32 (m, 14H, CH_2), 1.33–1.41 (m, 4H, CH_2), 1.42–1.51 (m, 4H, CH_2), 1.70–1.82 (m, 4H, CH_2), 2.09–2.19 (m, 2H, CH_2), 4.18 (t, 2H, J = 7.8 Hz, NCH_2), 5.02 (t, 2H, J = 7.6 Hz, N^+CH_2), 7.23 (ddd, 1H, J = 8.3, 7.3, 1.1 Hz, H8-Q), 7.34 (dd, 1H, J = 8.3, 0.6 Hz, H9-Q), 7.36–7.48 (m, 4H, H7-Q, *m*,*p*-Ph), 7.63 (dd, 1H, J = 8.4, 1.0 Hz, H6-Q), 8.06 (dd, 2H, J = 8.2, 1.3 Hz, *o*-Ph), 8.31 (dd, 1H, J = 8.0, 6.1 Hz, H5-Py), 8.58 (d, 1H, J = 8.0 Hz, H4-Py), 9.10 (s, 1H, H2-Py), 9.87 (d, 1H, J = 6.1 Hz, H6-Py). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C: 14.16, 14.17, 22.71, 22.74, 26.39, 27.17, 27.40, 29.19, 29.26, 29.32, 29.43, 29.44, 29.64, 31.58, 31.89, 31.94, 42.34, 63.22, 116.36, 119.10, 120.16, 121.45, 123.89, 128.12, 128.55, 129.06, 129.16, 130.00, 130.40, 132.10, 132.46, 136.61, 144.05, 144.31, 146.61, 146.66, 154.83. MS (MALDI TOF) = 591 [M − Br$^-$]$^+$. Found: C, 69.56; H, 7.61; N, 8.49; Br, 11.73. $C_{39}H_{51}N_4OBr$ requires: C, 69.73; H, 7.65; N, 8.34; Br, 11.89%.

*5.1.3.15. 1-Nonyl-3-(5-nonyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)pyridinium iodide (**3o**).* Yield 75%. Yellow powder, mp 96–99 °C (dec.). IR (ν_{max}, cm^{-1}, Nujol mull): 1649, 1610, 1487, 1408, 1343, 1301, 1255, 1198, 1168, 1118, 1072, 968, 923, 754, 695, 671. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.82–0.92 (m, 6H, CH_3), 1.20–1.32 (m, 14H, CH_2), 1.33–1.42 (m, 4H, CH_2), 1.42–1.52 (m, 4H, CH_2), 1.72–1.82 (m, 4H, CH_2), 2.10–2.20 (m, 2H, CH_2), 4.18 (t, 2H, J = 7.8 Hz, NCH_2), 4.92 (t, 2H, J = 7.7 Hz, N^+CH_2), 7.28 (dd, 1H, J = 8.0, 7.3 Hz, H8-Q), 7.34 (d, 1H, J = 8.0 Hz, H9-Q), 7.37–7.49 (m, 4H, H7-Q, *m*,*p*-Ph), 7.73

(d, 1H, J = 8.3 Hz, H6-Q), 8.07 (dd, 2H, J = 7.0 Hz, *o*-Ph), 8.30 (ddd, 1H, J = 8.0, 6.0, 0.5 Hz, H5-Py), 8.58 (d, 1H, J = 8.0 Hz, H4-Py), 9.10 (br s, 1H, H2-Py), 9.59 (d, 1H, J = 6.0, H6-Py). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_c: 14.21, 14.22, 22.76, 22.79, 26.43, 27.22, 27.45, 29.19, 29.29, 29.37, 29.46, 29.50, 29.69, 31.32, 31.93, 31.98, 42.41, 63.64, 116.43, 119.40, 120.35, 121.35, 124.23, 128.19, 128.63, 129.07, 129.24, 130.04, 130.43, 132.12, 132.76, 136.51, 144.04, 144.28, 146.12, 146.86, 154.89. MS (MALDI TOF) = 591 $[M - I^-]^+$. Found: C, 65.25; H, 7.10; N, 7.80; I, 17.60. $C_{39}H_{51}N_4OI$ requires: C, 65.17; H, 7.15; N,7.79; I, 17.66%.

*5.1.3.16. 1,4-Bis{3-(3-phenylimidazo[1,5-a]quinoxalin-4(5H)-on-1-yl)pyridinium}butane dibromide (**4a**).* Yield 51%. White powder, mp 273–275 °C (dec.). IR (ν_{max}, cm^{-1}, KBr): 3049, 2917, 1670, 1617, 1485, 1447, 1424, 1399, 1328, 1302, 1275, 1161, 785, 756, 746, 698, 679, 666. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.14 (br s, 4H, $NCH_2\underline{CH_2}$), 4.82 (br s, 4H, $N\underline{CH_2}CH_2$), 6.84 (ddd, 2H, J = 8.4, 7.3, 1.1 Hz, H8-Q), 7.17 (d, 2H, J = 8.4 Hz, H9-Q), 7.31 (d, 2H, J = 8.1, 7.3 Hz, H7-Q), 7.35 (dd, 2H, J = 8.1, 1.1 Hz, H6-Q), 7.41–7.53 (m, 6H, ArH), 8.18 (dd, 4H, J = 8.4, 1.4 Hz, *o*-Ph), 8.39 (dd, 2H, J = 8.1, 6.0 Hz, H5-Py), 8.94 (d, 2H, J = 8.1 Hz, H4-Py), 9.36 (d, 2H, J = 6.0 Hz, H6-Py), 9.64 (s, 2H, H2-Py), 11.62 (s, 2H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ_c: 27.17, 60.46, 116.83, 117.57, 120.00, 120.51, 122.12, 127.52, 127.77, 128.33, 128.47, 129.43, 129.68, 131.59, 132.24, 137.66, 144.13, 145.02, 145.48, 146.00, 154.85. MS (MALDI TOF) = 813 $[M - Br^-]^+$, 811 $[M - Br^-]^+$, 731 $[M4- HBr - Br^-]^+$. Found: C, 61.73; H, 4.01; N, 12.70; Br, 17.78. $C_{46}H_{36}N_8O_2Br_2$ requires: C, 61.89; H, 4.06; N,12.55; Br, 17.90%.

*5.1.3.17. 1,6-Bis{3-(3-phenylimidazo[1,5-a]quinoxalin-4(5H)-on-1-yl)pyridinium}hexane dibromide (**4b**).* Yield 55%. White powder, mp 321–323 °C (dec.). IR (ν_{max}, cm^{-1}, KBr): 3035, 2950, 2918, 2874, 2839, 2744, 2674, 1665, 1615, 1484, 1448, 1397, 1328, 1302, 1276, 1259, 1155, 812, 783, 765, 697, 671. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 1.42–1.48 (m, 4H, $N(CH_2)_2\underline{CH_2}$), 1.98–2.07 (m, 4H, $NCH_2\underline{CH_2}CH_2$), 4.73 (t, 4H, J = 7.5 Hz, $N\underline{CH_2}(CH_2)_2$), 6.96–7.02 (m, 2H, H8-Q), 7.27 (d, 2H, J = 8.4 Hz, H9-Q), 7.37–7.52 (m, 10H, ArH), 8.17–8.21 (m, 4H, *o*-Ph), 8.40 (dd, 2H, J = 8.1, 6.2 Hz, H5-Py), 8.98 (d, 2H, J = 8.1, H4-Py), 9.37 (d, 2H, J = 6.2 Hz, H6-Py), 9.66 (s, 2H, H2-Py), 11.67 (s, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ_c: 24.85, 30.32, 61.10, 116.94, 117.65, 119.94, 120.69, 122.30, 127.75, 127.76, 128.30, 128.47, 129.46, 129.79, 131.63, 132.25, 137.79, 144.19, 145.09, 145.44, 145.80, 154.89. MS (MALDI TOF) = 841 $[M - Br^-]^+$, 839 $[M - Br^-]^+$, 759 $[M - HBr - Br^-]^+$. Found: C, 62.50; H, 4.53; N, 12.32; Br, 17.16. $C_{48}H_{40}N_8O_2Br_2$ requires: C, 62.62; H, 4.38; N,12.17; Br, 17.36%.

*5.1.3.18. 1,3-Bis{3-(5-hexyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)pyridinium}xylene dibromide (4**c**).* Yield 72%. White powder, mp 252–254 °C (dec.). IR (ν_{max}, cm^{-1}, KBr): 3050, 3001, 2951, 2927, 2853, 1663, 1609, 1589, 1486, 1450, 1446, 1402, 1371, 1339, 1320, 1296, 1255, 1195, 1178, 1112, 987, 752, 698, 671. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.90 (t, 6H, J = 6.6 Hz, CH_3), 1.25–1.38 (m, 8H, CH_2), 1.38–1.48 (m, 4H, CH_2), 1.65–1.77 (m, 4H, CH_2), 4.00–4.15 (m, 4H, NCH_2), 6.29 (s, 4H, CH_2-xyl), 6.96 (dd, 2H, J = 7.7, 7.5 Hz, H8-Q), 7.15–7.45 (m, 13H, ArH), 7.74 (d, 2H, J = 7.7 Hz, H4,6-xyl), 7.94 (d, 4H, J = 7.5 Hz, *o*-Ph), 8.13 (br dd, 2H, J = 7.2, 6.6, H5-Py), 8.31 (d, 2H, J = 8.1, H4-Py), 8.50 (s, 1H, H2-xyl), 9.63 (br s, 2H, H2-Py), 9.93 (br d, 2H, J = 5.3, H6-Py). ^{13}C NMR (150.6 MHz, $CDCl_3$) δ_c: 14.18, 22.79, 26.91, 27.40, 31.68, 42.44, 62.25, 116.22, 119.22, 120.10, 121.25, 123.91, 128.12, 128.58, 129.16, 129.30, 130.05, 130.28, 130.65, 131.39, 131.83, 132.15, 132.42, 133.93, 136.44, 144.60, 144.94, 145.97, 146.67, 154.70. MS (MALDI TOF) = 1029 $[M - Br^-]^+$, 1027 $[M - Br^-]^+$, 947 $[M - HBr - Br^-]^+$. Found: C, 67.00; H, 5.41; N, 10.22; Br, 14.48. $C_{62}H_{60}N_8O_2Br_2$ requires: C, 67.15; H, 5.45; N, 10.10; Br, 14.41%.

*5.1.3.19. 1,3-Bis{3-(5-nonyl-3-phenylimidazo[1,5-a]quinoxalin-4-on-1-yl)pyridinium}xylene dibromide (**4d**).* Yield 68%. White powder, mp 242–245 °C (dec.). IR (ν_{max}, cm^{-1}, Nujol mull): 3200–2500, 1666, 1610, 1589, 1524, 1485, 1403, 1297, 1258, 1169, 1114, 836, 783, 752, 721, 701, 672. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.86 (t, 6H, J = 6.8 Hz, CH_3), 1.20–1.45 (m, 24H, CH_2), 1.70–1.85 (m, 4H, CH_2), 4.10 (t, 4H, J = 6.8 Hz, NCH_2), 6.29 (s, 4H, CH_2-xyl), 7.04 (dd, 2H, J = 7.5, 7.5 Hz, H8-Q), 7.22–7.35 (m, 10H, ArH), 7.40 (dd, 1H, J = 7.8, 7.8 Hz, H5-xyl), 7.50 (d, 2H, J = 8.5 Hz, H9-Q or H6-Q), 7.74 (d, 2H, J = 7.2 Hz, H4,6-xyl), 7.99 (d, 4H, J = 7.2 Hz, *o*-Ph), 8.13 (dd, 2H, J = 7.8, 5.8 Hz, H5-Py), 8.38 (d, 2H, J = 7.8 Hz, H4-Py), 8.54 (s, 1H, H2-xyl), 9.69 (s, 2H, H2-Py), 10.02 (br d, 2H, J = 5.8 Hz, H6-Py). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ_c: 14.25, 22.81, 27.25, 27.45, 29.39, 29.52, 29.73, 32.00, 42.44, 64.14, 116.18, 119.19, 120.09, 121.26, 123.87, 128.10, 128.53, 129.12, 129.24, 130.04, 130.28, 130.61, 131.34, 131.89, 132.18, 132.34, 133.97, 136.46, 144.59, 144.95, 145.96, 146.62, 154.70. MS (MALDI TOF) = 1113 $[M - Br^-]^+$, 1111 $[M - Br^-]^+$, 1031 $[M - HBr - Br^-]^+$. Found: C, 68.56; H, 6.21; N, 9.49; Br, 13.23. $C_{68}H_{72}N_8O_2Br_2$ requires: C, 68.45; H, 6.08; N, 9.39; Br, 13.39%.

*5.1.4. 1,10-Bis-{3-phenyl-1-(pyridin-3-yl)imidazo[1,5-a]quinoxalin-4-on-5-yl}decane (**5**)*

A solution of β-picolylamine (0.52 g, 4.8 mmol) in DMSO (3 mL) was added to a solution of 1,10-bis-(3-benzoyquinoxalin-2-on-1-yl) decane [26] (1.0 g, 1.6 mmol) in DMSO (7 mL). The reaction mixture was stirred for 8 h at 150 °C, and left overnight at room temperature, while the crystals of the product were precipitated, collected by suction filtration, washed with EtOH (10 mL) and dried. The yield was 60%. White powder, mp 235–237 °C (DMSO). IR (ν_{max}, cm^{-1}, KBr): 3052, 2923, 2852, 1656, 1610, 1592, 1567, 1499, 1485, 1469, 1447, 1394, 1336, 1323, 1299, 1252, 1183, 1120, 1025, 969, 745, 717, 692, 671. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.20–1.50 (m, 12H, CH_2), 1.70–1.85 (m, 4H, $NCH_2\underline{CH_2}$), 4.22 (t, 4H, J = 7.9 Hz, NCH_2), 6.97 (dd, 2H, J = 7.8, 6.5 Hz, H8-Q), 7.26–7.42 (m, 8H, ArH), 7.46 (d, 4H, J = 7.7, 7.1 Hz, *m*-Ph), 7.63 (dd, 2H, J = 7.8, 5.6 Hz, H5-Py), 8.12 (d, 4H, J = 7.2 Hz, *o*-Ph), 8.21 (d, 2H, J = 7.8 Hz, H4-Py), 8.84 (dd, 2H, J = 5.6, 0.7 Hz, H6-Py), 9.0 (d, 2H, J = 0.7 Hz, H2-Py). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_c: 27.17, 27.42, 29.44, 29.58, 42.09, 116.15, 118.05, 118.81, 122.41, 122.78, 123.88, 127.56, 128.07, 128.75, 128.76, 130.11, 130.64, 132.88, 137.33, 141.47, 146.12, 150.36, 151.07, 155.38. MS (MALDI TOF) = 815 $[MH]^+$. Found: C, 76.51; H, 5.75; N, 13.65. $C_{52}H_{46}N_8O_2$ requires: C, 76.64; H, 5.69; N,13.75%.

*5.1.5. 1^3,7^3-Diphenyl,-1^4,7^4-dioxa-1,7(1,5)-diimidazo[1,5-a] qunoxalina,-2(3,1),6(1,3)-dipyridina,-4(1,3)-benzenacycloheptadecaphane-2^1,6^1,-ylium dibromide (**6**)*

A solution of bisimidazo[1,5-*a*]quinoxaline **5** (0.24 g, 0.3 mmol) and *m*-dibromoxylene (77.8 mg, 0.3 mmol) in acetonitrile (240 mL) was refluxed for 24 h. The solvent was removed in vacuum and the residue was purified by recrystallization from $CHCl_3$ and then from the mixture $CHCl_3$:CH_3CN = 4:1. Yield 25%. White powder, mp 228–230 °C (dec.). IR (ν_{max}, cm^{-1}, Nujol mull): 3089, 2927, 2852, 1657, 1612, 1590, 1522, 1486, 1462, 1447, 1406, 1370, 1324, 1299, 1253, 1177, 1122, 1074, 1053, 993, 926, 813, 752, 696, 671. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 1.35–1.44 (m, 4H, CH_2), 1.45–1.52 (m, 8H, CH_2), 1.75–1.85 (m, 4H, CH_2), 4.05–4.15 (m, 4H, NCH_2), 6.03 (s, 4H, CH_2-xyl), 6.66 (dd, 2H, J = 8.3, 7.2 Hz, H8-Q), 7.09 (d, 2H, J = 8.3 Hz, H9-Q), 7.21 (dd, 2H, J = 8.3, 7.2 Hz, H7-Q), 7.30–7.40 (m, 6H, *m*,*p*-Ph), 7.44 (d, 2H, J = 8.4 Hz, H6-Q), 7.68 (dd, 1H, J = 7.8, 7.8 Hz, H5-xyl), 7.76 (s, 1H, H2-xyl), 7.78 (d, 2H, J = 8.0 Hz, H4,6-xyl), 7.98 (d, 4H, J = 7.6 Hz, *o*-Ph), 8.33 (dd, 2H, J = 7.7, 6.3 Hz, H5-Py), 8.60 (br s, 2H, H4-Py), 9.48 (br d, 2H, J = 6.3 Hz, H6-Py), 9.50 (s, 2H, H2-Py). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ_c: 24.79, 26.67, 27.13, 41.66, 63.30, 116.27, 118.08, 119.29, 121.01, 122.00, 127.36, 127.50, 128.33, 128.49, 129.16, 129.58, 129.80, 129.98, 130.98, 131.62, 131.90, 134.90, 136.81,

144.08, 144.37, 145.27, 146.00, 154.16. MS (MALDI TOF) = 999 $[M - Br^-]^+$, 997 $[M - Br^-]^+$, 917 $[M - HBr - Br^-]^+$. Found: C, 66.70; H, 4.96; N, 10.45; Br, 14.85. $C_{60}H_{54}N_8OBr_2$ requires: C, 66.79; H, 5.04; N,10.39; Br, 14.81%.

5.2. Biological evaluation

5.2.1. Antibacterial and antifungal activity

The *in vitro* antibacterial and antifungal activity of the imidazo[1,5-*a*]quinoxaline derivatives were investigated against several pathogenic representative Gram-negative bacteria (*P. aeruginosa* 9027, *E. coli* F-50), Gram-positive bacteria (*S. aureus* 209p, *B. cereus* 8035), moulds (*A. niger* BKMF-1119, *T. mentagrophytes* var. gypseum 1773) and yeast (*C. albicans* 885-653). Minimal inhibitory concentrations (MICs) were estimated by conventional dilution methods for bacteria and fungi [27,28]. The antibacterial and antifungal assays were performed in nutrient broth (bacteria 3×10^5 cfu/ml) and Sabouraud dextrose broth (fungi $2 \times 10^{3-4}$ cfu/ml). Ciprofloxacin, Ofloxacin, Norfloxacin, Clotrimazole and Amphotericin B were used as standard drugs. Positive growth control and standard drug controls were also run simultaneously. The MICs were defined as the lowest concentrations that showed no growth and were recorded by visual observation in every 24 h during 5 days for bacteria and after incubation during 14 days for fungi. The bactericidal and fungicidal activities were determined as follows. Assay tubes were filled with 1 mL of test compound solution in nutrient agar. Concentrations of test compounds were varied from 5 to 500 μg/ml. Normal saline broth (bacteria 3×10^5 cfu/ml), 1 mL was added to the tubes and kept for 4 h. The inocula were prepared by transferring the broth onto Petri plates containing meal-peptone agar. Petri plates were incubated at 37 °C and minimum bactericidal concentration (MBC) recorded as the test compound dilution affecting the total cell death. For fungicidal activity determination the tubes with the test compounds and fungi were incubated at 26 °C for 6 h. The inocula were prepared in Sabouraud dextrose broth and incubated at 26 °C.

5.2.2. Toxicity of the compounds studied

Toxicity tests were carried out by the intraperitoneal introductions of imidazo[1,5-*a*]quinoxaline derivative in aqueous solutions with the addition of 0.2% of the twin-80 in acute tests on white outbreed mice of both sexes with the mass of 18–22 g. The observation period was 72 h. Average lethal doses were used – LD50 as the criteria of toxicity. To measure these values each compound was introduced to 5 groups of mice (10 mice per dose; $n = 50$). The results were processed using the program ToxCalc™ v.5.0.23E (Tidepool Scientific Software; USA) [29].

Acknowledgements

This work was supported by the Russian Foundation for Basic Research (Grant No.10-03-00413-a) and Federal Agency of Science and Innovation (the state contract № 02.740.11.0633).

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.05.038. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] F. Baffert, C.H. Regnier, A. De Pover, C. Pissot-Soldermann, G.A. Taveres, F. Blasco, J. Brueggen, P. Chene, P. Drueckes, D. Erdmann, P. Furet, M. Gerspacher, M. Lang, D. Ledieu, L. Nolan, S. Ruetz, J. Trappe, E. Vanghrevelinghe, M. Wartmann, L. Wyder, F. Hofmann, T. Radimerski, Mol. Cancer Ther. 9 (2010) 1945.
[2] S.B. Lee, Y.I. Park, M.S. Dong, Y.D. Gong, Bioorg. Med. Chem. Lett. 20 (2010) 5900–5904.
[3] E.S.H. El-Ashry, A.A.H. Abdel-Rahman, N. Rashed, H.A. Rasheed, Pharmazie 54 (1999) 893–897.
[4] L.E. Seitz, W.J. Suling, R.C. Reynolds, J. Med. Chem. 45 (2002) 5604–5606.
[5] A.A. Abu-Hashem, M.A. Gouda, F.A. Badria, Eur. J. Med. Chem. 45 (2010) 1976–1981.
[6] S.N. Khattab, S.Y. Hassan, A.A. Bekhit, A.M. El Massry, V. Langer, Eur. J. Med. Chem. 45 (2010) 4479–4489.
[7] M.R. Myers, W. He, B. Hanney, N. Setzer, M.P. Maguire, A. Zulli, G. Bilder, H. Galzcinski, D. Amin, S. Needle, A.P. Spada, Bioorg. Med. Chem. Lett. 13 (2003) 3091–3095.
[8] B. Dietrich, U. Diederchsen, Eur. J. Org. Chem. (2005) 147–153.
[9] P.K. Sasmal, R. Majumdar, R.R. Dighe, A.R. Chakravarty, Dalton Trans. 39 (2010) 7104–7113.
[10] J.L. Sessler, H. Maeda, T. Mizuno, V.M. Lynch, H. Furuta, Chem. Commun. (2002) 862–863.
[11] M. Negwer, H.G. Scharnow, Organic Chemical Drugs and Their Synonyms, Wiley-VCH, Weinheim, Germany, 2001.
[12] R.E. Ten Brink, E.J. Jacobsen, J.B. Hester, L.L. Skaletzky, WO 9317025 PCT, 1993.
[13] F. Waetjen, H.C. Hansen, WO 9107407 PCT, 1991.
[14] F. Watjen, H.C. Hansen, EP 368652, 1990.
[15] R.E. TenBrink, E.J. Jacobsen, P.B. Gammill, US 5541324, 1996.
[16] E.J. Jacobsen, R.E. TenBrink, L.S. Stelzer, K.L. Belonga, D.B. Carter, H.K. Im, W.B. Im, V.H. Sethy, A.H. Tang, P.F. VonVoigtlander, J.D. Petke, J. Med. Chem. 39 (1996) 158–175.
[17] R.E. TenBrink, W.B. Im, V.H. Sethy, A.H. Tang, D.B. Carter, J. Med. Chem. 37 (1994) 758–768.
[18] E.J. Jacobsen, L.S. Stelzer, K.L. Belonga, D.B. Carter, W.B. Im, V.H. Sethy, A.H. Tang, P.F. VonVoigtlander, J.D. Petke, J. Med. Chem. 39 (1996) 3820–3836.
[19] E.J. Jacobsen, L.S. Stelzer, R.E. TenBrink, K.L. Belonga, D.B. Carter, H.K. Im, W.B. Im, V.H. Sethy, A.H. Tang, P.F. VonVoigtlander, J.D. Petke, W.Z. Zhong, J.W. Mickelson, J. Med. Chem. 42 (1999) 1123–1144.
[20] K. Shaw, WO 9207853 PCT, 1992.
[21] D.D. Davey, P.W. Erhardt, E.H. Cantor, S.S. Greenberg, W.R. Ingebretsen, J. Wiggins, J. Med. Chem. 34 (1991) 2671–2677.
[22] J. Ohmori, M. Shimizu-Sasamata, M. Okada, S. Sakamoto, J. Med. Chem. 40 (1997) 2053–2963.
[23] D.D. Davey, EP 400583, 1990.
[24] M.S. Malamas, Y. Ni, J.J. Erdey, U. Egerland, B. Langen, WO 2010138833 PCT, 2010.
[25] V.A. Mamedov, A.A. Kalinin, A.A. Balandina, I.Kh. Rizvanov, S.K. Latypov, Tetrahedron 65 (2009) 9412–9420.
[26] V.A. Mamedov, A.A. Kalinin, A.T. Gubaidullin, E.A. Gorbunova, I.A. Litvinov, Russ. J. Org. Chem. 42 (2006) 1532–1543.
[27] V.E. Semenov, A.D. Voloshina, E.M. Toroptzova, N.V. Kulik, V.V. Zobov, R.K. Giniyatullin, A.S. Mikhailov, A.E. Nikolaev, V.D. Akamsin, V.S. Reznik, Eur. J. Med. Chem. 41 (2006) 1093–1101.
[28] V.E. Semenov, A.S. Mikhailov, A.D. Voloshina, N.V. Kulik, A.D. Nikitashina, V.V. Zobov, S.V. Kharlamov, S.K. Latypov, V.S. Reznik, Eur. J. Med. Chem. 46 (2011) 4715–4724.
[29] H.L. Brooks, Pesticide Background Statements , in: Intecticides, vol. IV, Department of Agriculture, Agriculture Handbook, No 685, Forest Service, U.S., 1989.