Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 41 (2006) 1093–1101
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://france.elsevier.com/direct/ejmech

Preliminary communication

Antibacterial and antifungal activity of acyclic and macrocyclic uracil derivatives with quaternized nitrogen atoms in spacers

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

A.E. Arbuzov Institute of Organic and Physical Chemistry of the Russian Academy of Sciences, Kazan, 420088, Arbuzov str. 8, Russian Federation

Received 27 July 2005; received in revised form 23 March 2006; accepted 27 March 2006
Available online 09 June 2006

Abstract

The reactions of 1,3-bis(α,ω-bromoalkyl)-6-methyluracils with 1,3-bis(α,ω-ethylaminoalkyl)-6-methyluracils or 1,3-bis(bromopentyl)thymine with butylamine afforded pyrimidinophanes containing one or two uracil units and nitrogen atoms in bridging polymethylene chains. In some cases individual geometric isomers of pyrimidinophanes differing in the mutual arrangement of the carbonyl and methyl groups at different pyrimidine rings were isolated. Quaternization of the bridging nitrogen atom with *o*-nitrobenzyl bromide, benzyl bromide, *n*-decyl bromide gave rise to water-soluble pyrimidinophanes which were evaluated for their antibacterial and antifungal activity. The arrangement of the carbonyl groups in macrocycles doesn't affect the activity. Antibacterial and antifungal activity of pyrimidinophanes increases with the increase of polymethylene $N_{(pyr)}$-N-chain length and dramatically increases upon the introduction of *n*-decyl substituent at nitrogen atoms in spacers. Pyrimidinophanes with 5 and 6 methylene groups in $N_{(pyr)}$-N-chain and *n*-decyl substituent showed significant bacteriostatic, fungistatic, bactericidal, fungicidal activity which comparable with standard antibacterial and antifungal drugs. Acyclic counterpart demonstrated the highest activity against fungi. Toxicity of more effective pyrimidinophanes was determined for mice and *Daphnia magna Straus*.

Keywords: Pyrimidinophanes; Uracils; Quaternization; Antibacterial activity; Antifungal activity; Toxicity

1. Introduction

At present time the synthesis of macrocyclic compounds containing pyrimidine rings, pyrimidinophanes, is rapidly developing field of pyrimidines chemistry. Since first reports concerning pyrimidinophanes synthesis [1,2] diverse macrocyclic structures have been prepared. These macrocycles can contain different number of pyrimidine units linked to each other by hydrocarbon or polyether spacers through either the N(1) and N(3) atoms or carbon atoms of pyrimidine rings or substituents at pyrimidine rings [3–13]. In most of known pyrimidinophanes uracil derivatives fragments were introduced as pyrimidine units because the chemical features of uracils allow to vary the structure of the macrocyclic compounds synthesized.

It was supposed that pyrimidine derivative (a building unit of some coenzymes) being contained in a molecule which also includes a fragment exhibiting a specific activity with respect to certain biotarget, would increase the specificity of the molecule relative to the target. This approach was successfully applied to development of adrenolytics containing pyrimidine unit [14] and a new class of cholinesterase inhibitors based on uracil derivatives [15–20]. Unique specificity of acyclic 3-mono- or 1,3-bis[(trialkylamino)alkyl]uracil derivatives with respect to acetylcholinesterase is explained by "appropriate" mutual arrangement of uracil and tetralkylammonium fragments. It is obvious that this "appropriate" arrangement of uracil unit allow the inhibitor to bind more strongly with the molecule of acetylcholinesterase.

We proposed that an efficacy of specific fragment connecting with pyrimidinophane consisting of uracil units would dramatically increase due to additional binding with biotarget. It can be caused by more rigid framework of macrocyclic compounds in comparison with their acyclic counterparts and pro-

* Corresponding author.
E-mail address: sve@iopc.kcn.ru (V.E. Semenov).

0223-5234/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.03.030

vided that the macrocycle have a complementarity with biotarget particularly with enzyme. It is possible that the pyrimidinophanes can exhibit activity by other mechanism due to macrocyclic framework as specific fragment.

To our knowledge, in spite of advances in pyrimidinophanes chemistry biological activity data for these compounds absolutely have not been disclosed so far. The possible explanation is insolubility of almost all known pyrimidinophanes in polar solvents, particularly, in water, which prevents their biological properties study. Earlier we have reported the synthesis of first pyrimidinophanes which contain two 6-methyluracil fragments linked to each other by polymethylene chains with nitrogen atom [13]. We isolated individual geometric isomers with cis- or trans-arrangement of the carbonyl and methyl groups at different pyrimidine rings. Reaction of the isomeric macrocycles with substituted benzyl bromide gave rise to water-soluble pyrimidinophanes with quaternary ammonium in bridges. For macrocyclic compounds of this type a wide screening of various kinds of biological activity is possible.

It is widely known that quaternary ammonium salts in combination with lipophilic residues display unspecific activity through interaction with cell walls and cell membranes of microorganisms. Use of the organic cations as disinfectants in agriculture, the 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 the mentioned above proposals and considering the pyrimidinophane framework as specific fragment available to interfere in main biochemical pathways (cell wall synthesis, DNA and RNA synthesis, protein formation) we have prepared a series of pyrimidinophanes as individual isomers and couple of isomers, quaternized them by benzyl and alkyl bromides, and this preliminary communication demonstrates the in vitro antibacterial and antifungal activity of the pyrimidinophanes, their toxicity, which have not been studied so far. Also we have synthesized and tested new type of pyrimidinophanes consisting of one uracil unit and the spacer with nitrogen atom which connects N_1 and N_3 of pyrimidine ring. Moreover, we tested the acyclic compounds having structural features of the examined pyrimidinophanes. The acyclic compounds are introduced in the screening for attempt to determine fragments responsible for antimicrobial activity and evaluate importance of ring-closure for efficacy. The objective of our study is to generate novel leads and novel active compounds and to optimize the structure to display the potent efficacy. At present time when new infectious diseases appear it becomes especially important and the search of quiet new agents is necessary.

2. Chemistry

Recently we reported the synthesis of pyrimidinophanes with nitrogen atom in spacers by reactions of 1,3-bis(α,ω-bromobutyl or pentyl)-6-methyluracils with 1,3-bis(α,ω-ethylaminobutyl or pentyl)-6-methyluracils [13]. These reactions imply the formation of isomers and we succeeded in isolating individual geometric isomers of pyrimidinophanes by column chromatography. The isomeric pyrimidinophanes differ one from another by the mutual arrangement of the carbonyl and methyl groups at different pyrimidine rings— *cis*- and *trans*-isomers **1a** and **1b**, **2a** and **2b**. The structure of these macrocycles was confirmed by X-ray [13]. As the result the formulas with *cis*- (**1a** and **2a**) or *trans*- (**1b** and **2b**) arrangement of the carbonyl groups were unambiguously assigned to concrete isolated macrocycles (Fig. 1).

The same synthetic protocol was used for the preparation of pyrimidinophanes with 6 methylene groups in $N_{(pyr)}$-N-chain. Interaction of 1,3-bis(6-bromohexyl)-6-methyluracil (**4**) with 1,3-bis(6-ethylaminohexyl)-6-methyluracil (**5**), the latter being obtained from **4** and ethylamine, gave isomeric pyrimidinophanes **3a** and **3b** (Scheme 1). However, in this case we didn't succeed to isolate individual geometric isomers of macrocycles and obtained a mixture of macrocycles **3a** and **3b** with *trans*- and *cis*- arrangement of the carbonyl and methyl groups. Water-soluble pyrimidinophanes **6** [13], **7a**, **7b**, **8** [13] and **9** were prepared by quaternization of the nitrogen atoms in bridges with *o*-nitrobenzyl, benzyl or *n*-decyl bromides in acetonitrile according to established procedure. The reaction of **3a** and **3b** mixture with benzyl bromide or *n*-decyl bromide afforded the mixture of isomers **10a** and **10b**, **11a** and **11b**, respectively. Synthesized isomeric pyrimidinophanes with quaternary ammonium in spacers are presented in Fig. 2. These macrocycles are well soluble in water in wide region of concentrations enough for screening.

Acyclic compounds **12**–**14** were studied for comparison. These reference compounds may be considered as acyclic counterparts of pyrimidinophanes. Ammonium salt (**15**) is used for simulating of cationic head in acyclic and macrocyclic compounds (Fig. 3). Synthetic procedures of **12**–**14** are the same as that for pyrimidinophanes and Scheme 2 shows the preparation of compounds **12** and **13** which can be considered as acyclic "half" of the macrocycles. In all cases quaternization of the nitrogen atoms with benzyl or *n*-decyl bromides is followed by the preparation of neutral ligands **18a**, **18b** and **19**. Neutral ligands in their turn were afforded by the reactions of corresponding bromides with corresponding amines.

1-Mono- or 1,3-bis(aminoalkyl)uracils were prepared by amination of 1-mono- or 1,3-bis(bromoalkyl)uracils with a

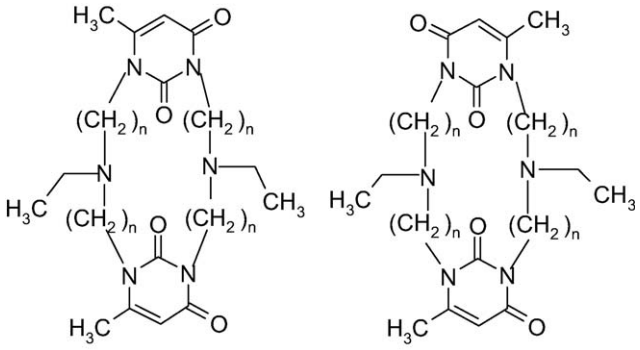
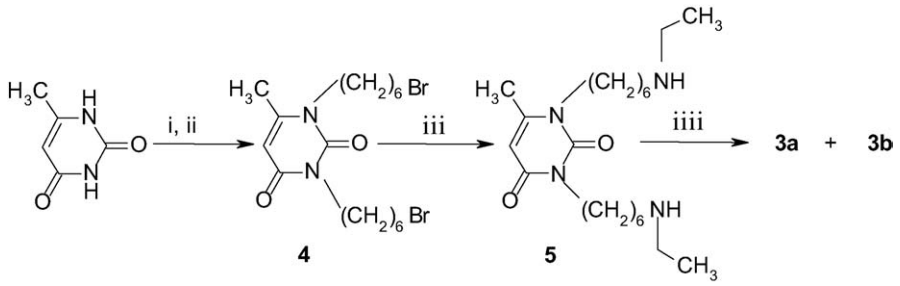


Fig. 1. Isomeric pyrimidinophanes with *cis*- (**1a**, **2a**, **3a**) and *trans*- (**1b**, **2b**, **3b**) arrangement of the carbonyl groups.



Scheme 1. Synthesis of **3a** and **3b**, reagents and conditions: (i) Na, *n*-BuOH, reflux, 15 h; (ii) $Br(CH_2)_6Br$, DMF, 50–60 °C, 10 h; (iii) 40-fold excess of NH_2Et, i-PrOH, room temperature, 7 days; (iiii) K_2CO_3, CH_3CN, 60–70 °C, 12 h.

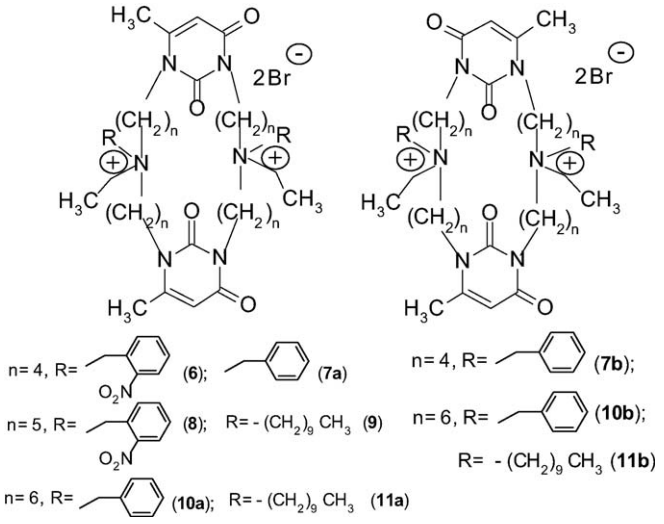


Fig. 2. Isomeric pyrimidinophanes with quaternized N in spacers.

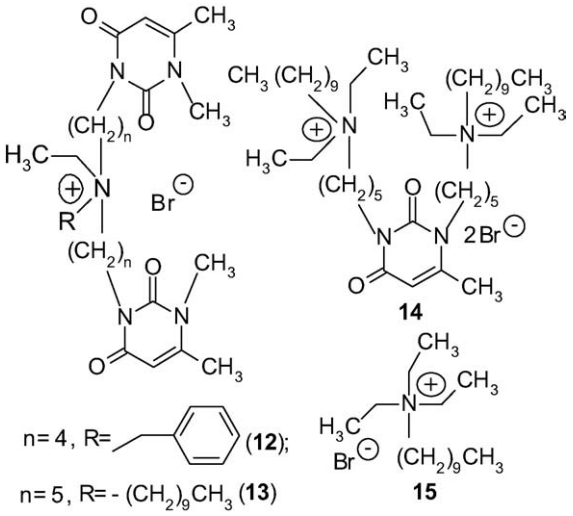
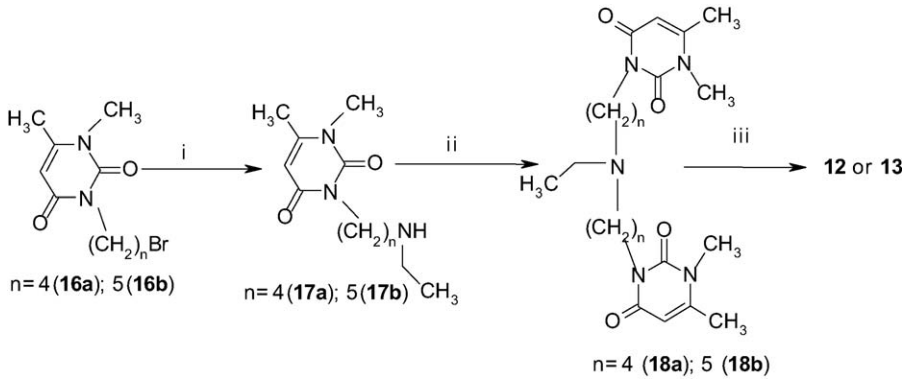


Fig. 3. Acyclic counterparts of pyrimidinophanes **12–14** and model compound **15**.

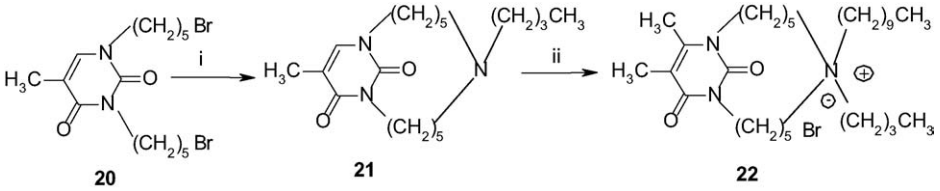
considerable excess of appropriate amine (Schemes 1 and 2). When 1,3-bis(bromoalkyl)uracil/amine ratio is 1:1–1:3 as 1(3)-mono- or 1,3-bis(aminoalkyl)uracils as macrocyclic product can be formed. Particularly preparation of macrocycle **21** is depicted in Scheme 3. We isolated pyrimidinocyclophane **21** from the reaction mixture of *n*-butylamine and 1,3-bis(bromopentyl)thymine (**20**) and the following quaternization with *n*-decyl bromide gave macrocycle **22**. These pyrimidinocyclophanes have interesting conformational and spectral features, which will be discussed elsewhere.

3. Biological results and discussion

The in vitro antibacterial and antifungal activity of the pyrimidinophanes **6**, **7a,b**, **8**, **9**, **10a,b**, **11a,b**, **22** was investigated against several pathogenic representative Gram-negative bacteria (*Pseudomonas aeruginosa* 9027 and *Escherichia coli* F-50), Gram-positive bacteria (*Staphylococcus aureus* 209p, *Bacillus subtilis* 6633 and *Enterococcus faecalis* ATCC 8043), pathogenic fungi (*Aspergillus niger* BKMF-1119, *Trichophyton mentagrophytes var. gypseum* 1773 and *Aspergillus fumigatus* AF-27) and yeast (*Candida Albicans* 885-653). Results are presented in Tables 1 and 2. It is necessary to note that couples of isomeric macrocycles **10a** and **10b**, **11a** and **11b** were screened as mixtures because we didn't succeed in isolating of individual geometric isomers of macrocycles **3a** and **3b** and introduced the mixture of them in the reaction of quaternization by benzyl bromide or *n*-decyl bromide. Data of the tables can be related to structural features of the compounds, particularly *trans*- or *cis*-arrangement of the carbonyl groups, aliphatic or aromatic substituent at the nitrogen atoms in bridges, length of spacer, cyclic or acyclic geometry. The screened compounds exhibit bacteriostatic and fungistatic activity in the regions of concentrations 0.98–5000 μg/ml and 30–2500 μg/ml, respectively. As evident from antibacterial data pyrimidinophanes **1a**, **6**, **7a,b**, **8** are showing a slight bacteriostatic activity against Gram-positive bacteria *S. aureus* and *B. subtilis* but absolutely are not active against Gram-negative bacteria *E. coli* and *P. aeruginosa* and moulds *A. niger* and *T. mentagrophytes*. Quaternization of macrocycle **1a** by *o*-nitrobenzyl bromide don't affect the activity of the resulting pyrimidinophane **6**. However, quaternization of macrocycle **1a** by benzyl bromide which hasn't electron withdrawing substituent gave macrocycle **7a** with dramatic decrease of MIC values. Pyrimidinophanes **7a** and **7b** with *cis*- and *trans*-arrangement of the carbonyl groups at different pyrimidine rings, respectively, exhibit the same antibacterial activity and it shows that the arrangement of the carbonyl groups doesn't affect the activity. Also in this case cyclic structure of the isomers **7a** and **7b** doesn't influence their activity because the acyclic counterpart **12** with one nitrogen atom



Scheme 2. Synthesis of **12** and **13**, reagents and conditions: (i) 40-fold excess of NH_2Et, i-PrOH, room temperature, 3 days; (ii) **16a** or **16b**, K_2CO_3, CH_3CN, reflux, 12 h; (iii) 1.2-fold excess of benzyl bromide or twofold excess of *n*-decyl bromide, CH_3CN, reflux, 20 h.



Scheme 3. Synthesis of pyrimidinocyclophanes **21** and **22**, reagents and conditions: (i) threefold excess of *n*-$BuNH_2$, K_2CO_3, $TBA^{\cdot}HSO_4$, *n*-BuOH, 70–75 °C, 11 h; (ii) fourfold excess of *n*-decyl bromide, CH_3CN, reflux, 20 h.

Table 1
In vitro antibacterial and antifungal activity of pyrimidinophanes and acyclic counterparts[a]

	Minimal Inhibitory Concentration (MICs) μg/ml								
N°	Sa	Bs	Ec	Pa	Ef	An	Tm	Af	Ca
1a	> 1000	> 1000	–	–	n.d.	–	–	n.d.	n.d.
6	> 1000	> 1000	–	–	n.d.	–	–	n.d.	n.d.
7a	312	625	–	–	n.d.	–	–	n.d.	n.d.
7b	312	625	–	–	n.d.	–	–	n.d.	n.d.
8	125	500	–	–	n.d.	–	–	n.d.	n.d.
9	0.98	7.8	62.5	>1000	500	250	125	> 1000	250
10a and **10b**	50	100	>1000	–	n.d.	–	–	n.d.	n.d.
11a and **11b**	0.98	1.95	62.5	500	156	250	125	250	250
12	625	> 1000	–	–	n.d.	–	–	n.d.	n.d.
13	78	156	> 1000	–	n.d	> 1000	625	n.d.	n.d.
14	1.56	7.8	12.5	–	312	62.5	31.3	125	62.5
15	2.44	50	156	–	625	10	500	> 1000	500
22	78	625	> 1000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ampicillin	0.13	625	3.13						
Clo							3.13	0.19	0.39
Cip				0.12	3.9				
Amph						20			

[a] The tests were performed in duplicate and repeated twice; n.d., not done; -, no activity was showed; Pa, *Pseudomonas aeruginosa;* Ec, *Escherichia coli*; Sa, *Staphylococcus aureus;* Ba, *Bacillus subtilis*; Ef, *Enterococcus faecalis*; An, *Aspergillus niger*; Af, *Aspergillus fumigatus;* Tm, *Trichophyton mentagrophytes*; Ca, *Candida albicans*; Clo, Clotrimazole; Cip, Ciprofloxacin; Amph, Amphotericin B.

which is quaternized by benzyl bromide shows MICs precisely twice as large as MICs of these pyrimidinophanes. Increase of the polymethylene $N_{(pyr)}$-N-chain length up to 5 methylene groups in macrocycle **8** and up to 6 methylene groups in mixture of isomeric pyrimidinophanes **10a** and **10b** dramatically increases antibacterial activity: MICs of pyrimidinophane **8** and mixture of the pyrimidinophanes **10a** and **10b** are threefold and sixfold, respectively, less than MICs of **7a** or **7b** with 4 methylene groups in $N_{(pyr)}$-N-chain.

It is obvious from the structure–activity profile of pyrimidinophanes that lipophilic *n*-decyl substituent at nitrogen atom in spacer greatly influence the antibacterial and antifungal activity. Macrocycle **9** and isomeric pyrimidinophanes **11a** and **11b** mixture show high activity against bacteria and fungi, and in this case there is the trend of an increase of the efficacy of the compounds with an increase of the polymethylene spacer length. In particular pyrimidinophanes **11a** and **11b** mixture exhibit the dramatic increase of the efficacy against *Bacillus*

Table 2
Bactericidal activity of pyrimidinophanes **9, 11a,b** and acyclic compounds **13–15**[a]

Number	*S. aureus*		*B. subtilis*		*E. aecalis*	
	MBC, μg/ml	Time, min	MBC, μg/ml	Time, min	MBC μg/ml	Time, min
9	500	30	4000	120	500	240
11a and **11b**	500	10	990	120	500	15
13	10^4	–	10^4	–	10^4	–
14	800	240	4000	240	300	240
15	1250	240	10^4	–	1000	240
Ampicillin	0.5	240	6250	240		
Cip					50	15

[a] MBC, minimum bactericidal concentration; Time, time of total cell death; -, no activity was showed at the concentration; Cip, Ciprofloxacin.

subtilis, Enterococcus faecalis and *Aspergillus fumigatus* compared with pyrimidinophane **9**.

Acyclic compound **14** which has the structure similar to pyrimidinophane **9** exhibits bacteriostatic activity lower than the macrocyclic counterpart but demonstrates the highest efficacy against fungi and yeast. Compound **13** is "half" of pyrimidinophane **9**; however, its activity is significantly lower than activity of macrocyclic and acyclic counterparts **9** and **14**. Importance of long alkyl chain substituent at nitrogen atom in spacer is confirmed by MICs values of the reference compound **15**. In fact, a long alkyl chain suggest detergent-like properties and unspecific mechanism of action. However, if discussed compounds simply acted by a non-specific detergent-like mechanism, much lipophilic pyrimidinocyclophane **22** would be the most active compound. Contrary, MICs of the macrocycle are the lowest for acyclic and macrocyclic uracil derivatives **9**, **11a** and **11b**, **13**, **14** with *n*-decyl substituent. It is evident that in vitro antibacterial and antifungal activity of the compounds depends not only on substituents at the cationic head but also the mutual arrangement of these substituents and uracil unit.

Macrocyclic and acyclic compounds with the highest bacteriostatic and fungistatic efficacy were further screened for their bactericidal and fungicidal activity (Tables 2 and 3). The screening data against Gram-positive bacteria and pathogenic fungi revealed that pyrimidinophane **9**, mixture of the isomeric pyrimidinophanes **11a** and **11b** have the best bactericidal properties and acyclic compound **14** have the best fungicidal properties. Meanwhile, model detergent-like compound **15** doesn't exhibit the bactericidal and fungicidal properties anyway. The antibacterial and antifungal efficacy of the screened compounds were compared with that showed by the standard antibacterial drugs ampicillin and ciprofloxacin, and antifungal drugs clotrimazole and amphotericin B. Pyrimidinophanes **9**, **11a** and **11b** display bacteriostatic activity against *E. coli* and *S. aureus* comparable to the reference drug ampicillin while their activity against *B. subtilis* is 300-fold more than that of ampicillin. The screening data against fungi revealed that pyrimidinophanes **9**, **11a** and **11b**, acyclic compound **14** show modest activity compared with that of the reference drugs.

Table 3
Fungicidal activity of pyrimidinophanes **9, 11a,b** and acyclic compounds **13–15**[a]

Number	*A. niger*		*T. mentagrophytes*		*C. albicans*	
	MFC μg/ml	Time min	MFC μg/ml	Time min	MFC μg/ml	Time min
9	500	180	250	180	250	180
11a and **11b**	500	90	250	120	250	180
13	10^4	–	2500	360	n.d.	n.d.
14	125	180	62.5	180	65	180
15	10^4	–	10^4	–	500	240
Clo	10^3	360	12.5	360	31.25	360
Amph	250	120				

[a] MFC, minimum fungicidal concentration; Time, time of total cell death; n. d., not done; -, no activity was showed at the concentration; Clo, Clotrimazole; Amph, Amphotericin B.

Table 4
Toxicity of pyrimidinophanes **6**, **8**, **9**, **11a** and **11b** on mice and Daphnia

Number	LC_{50}, *Daphnia magna*, mg/l	LD_{50}, mice, mg/kg
6	110.6 (94.5 ÷ 37.4)	13,6 (11.9 ÷ 15.5)
8	54.3 (46.8 ÷ 62.9)	9.5 (8.2 ÷ 11.0)
9	14.3 (12.1 ÷ 16.9)	23.4 (19.6 ÷ 25.1)
11a and **11b**	5.1 (4.4 ÷ 6.0)	18.8 (16.3 ÷ 21.6)

We have evaluated the toxicity of the pyrimidinophanes **6**, **8**, **9** and the mixture of isomeric pyrimidinophanes **11a** and **11b** in terms of lethal doses (LD_{50}) for mice and lethal concentrations (LC_{50}) for *Daphnia magna Straus*. Toxicity data are presented in Table 4. According to levels of ecological safety for *Daphnia magna* [22] pyrimidinophanes **6**, **8**, **9** can be considered as slightly toxic or practically non-toxic, and isomeric pyrimidinophanes **11a** and **11b** as moderately toxic compounds. According to levels of acute toxicity for mammals the pyrimidinophanes can be considered as slightly toxic and moderately toxic compounds. Toxicity data show that lethal doses for *Daphnia magna* dramatically decrease with the increase of activity of the pyrimidinophanes. On the contrary, for mammals lethal concentrations increase with the increase of activity.

4. Conclusions

We have described the antibacterial and antifungal activity of a series of pyrimidinophanes with one and two uracil units, *cis*- or *trans*-arrangement of carbonyl groups at different pyrimidine rings, with quaternized or not quaternized nitrogen atoms in the bridges and their acyclic counterparts. The screening data show that the activity depends on different structural features, particularly, lipophily of substituent at nitrogen atom in spacer, length of $N_{(pyr)}$-N-chain and relative orientation of the cationic head and uracil fragment. In some cases cyclic structure of the screened compounds increases the activity with respect to acyclic counterparts. However, acyclic compound **14** is more active against fungi than isostructural pyrimidinophanes. The mode of action of the screened compounds is not clear. Direct data show that a specific mechanism of action is lacking. It is obvious that uracil units are not responsible for the antibacterial and antifungal effect of the pyrimidinophanes and their acyclic counterparts; however, the appropriate arrangement of uracil unit relative to quaternary ammonium in spacer can significantly increase antibacterial and antifungal efficacy.

5. Experimental protocols

5.1. Chemistry

5.1.1. Materials

The ^{1}H NMR spectra were recorded on Bruker MSL-400 spectrometer with Me_4Si as the internal standard. The IR spectrum (in KBr) was recorded on a Vector 22 Fourier spectrometer (Bruker) under standard conditions. The mass spectra (EI) were obtained on a Finnigan MAT-212 mass spectrometer (70 eV). The melting points were measured on a Boetius hot-stage apparatus or in open capillary tubes. Thin layer chromatography was performed on Silufol-254 plates; visualization was carried out with UV light. For column chromatography, silica gel of 60 mesh from Fluka and neutral Al_2O_3 (activity II) were used. All solvents were dried according to standard protocols. Pyrimidinophanes **1a, 1b**, **2a, 2b**, **6**, **8** were prepared by known procedures [13]. Also known protocol was applied to synthesis of compounds **16a** [11] and **19** [15]. Compounds **16b**, **17a**, **17b** were obtained using established synthetic routes. *n*-Decyltriethylammonium bromide **15** was obtained with 87% yield and m.p. 124–127 °C by the reaction of NEt_3 with *n*-decyl bromide in CH_3CN (106–107 °C [22]).

5.1.2. Synthesis of pyrimidinophanes ***3a*** *and* ***3b***

*5.1.2.1. 1,3-Bis(6-bromohexyl)-6-methyluracil (**4**).* 1,6-Dibromohexane (300 g, 1.23 mol) was added with stirring to a suspension of disodium salt of 6-methyluracil (27 g, 0.159 mol) in DMF (250 ml) and the mixture was stirred at 55–65 °C for 8 h. The mixture was evaporated in a vacuum, and the residue was treated with chloroform to extract the reaction products. The precipitate was filtered off and the filtrate was concentrated to 10–20 ml and transferred to a column with Al_2O_3. The column was successively washed with petroleum ether and a 1:1 petroleum ether-ethyl ether mixture. From petroleum ether-ethyl ether fractions compound **4** was obtained in a yield of 11.2 g (16%) as oil; R_f 0.41 (5:1 ethyl ether-hexane as the eluent); ^{1}H NMR ($CDCl_3$); δ 1.34–1.90 (m, 16H, CH_2), 2.27 (s, 3H, $C_{6(pyr)}CH_3$), 3.41 (m, 4H, CH_2Br), 3.79 (t, $J = 8$ Hz, 2H, $N_{(pyr)}CH_2$), 3.94 (t, $J = 7.6$ Hz, 2H, $N_{(pyr)}CH_2$,), 5.56 (s, $C_{5(pyr)}H$); Anal. calcd for $C_{17}H_{28}Br_2N_2O_2$: C, 45.15; H, 6.24; Br 35.34; N, 6.19; found: C, 45.22; H, 6.26; Br 35.44; N, 6.36.

*5.1.2.2. 1,3-Bis(6-ethylaminohexyl)-6-methyluracil (**5**).* Compound **4** (5 g, 11.1 mmol) was added to a 20% $EtNH_2$ solution in *i*-PrOH (100 ml). The reaction mixture was kept at room temperature for 7 days and then concentrated in vacuo. A solution of MeONa, which was prepared from sodium (0.51 g, 22.2 mmol) in methanol (30 ml), was added to the residue. The solvent was evaporated in vacuo and the reaction product was extracted with ethyl ether (2 × 50 ml). The ether was distilled off to obtain compound **5** in a yield of 4 g (97%) as oil; R_f 0.09 (6:6:1 ethyl acetate/ethyl ether/diethyl amine as the eluent); IR (KBr) cm^{-1}: 1680, 1720 (CO), 3550–3200 (NH); ^{1}H NMR ($CDCl_3$): δ 1.12 (m, 6H, CH_3), 1.36–1.63 (m, 16H, CH_2), 2.26 (s, 3H, $C_{6(pyr)}CH_3$), 2.57–2.68 (m, 10H, $NHCH_2$), 3.80 (t, $J = 8$ Hz 2H, $N_{(pyr)}CH_2$), 3.90 (t, $J = 7.6$ Hz, 2H, $N_{(pyr)}CH_2$), 5.57 (s, $C_{5(pyr)}H$); Anal. calcd for $C_{21}H_{40}N_4O_2$: C, 66.28; H, 10.59; N, 14.72; found: C, 66.35; H, 10.66; N, 14.66.

*5.1.2.3. 18, 34-Dimethyl-8,26-diethyl-1,8,15,19,26,33-hexaazatricyclo[31,3,1,$1^{15,19}$]-octatriaconta-17,34-diene-16,36,37,38-tetraone (**3a**) and 18,36-dimethyl-8,26-diethyl-1,8,15,19,26,33-hexaazatricyclo[31,3,1,$1^{15,19}$]-octatriaconta-17,35-diene-16,34,37,38-tetraone (**3b**).* Potassium carbonate (4.0 g, 30.0 mmol) was added to a solution of dibromide **4** (4.88 g, 10.8 mmol) and diamine **5** (4.0 g, 10.5 mmol) in acetonitrile (100 ml) and the reaction mixture was stirred at 60–70 °C for 12 h. The precipitate was filtered off. The solution was concentrated to 10–15 ml and transferred to a column with SiO_2. The column was successively washed with ethyl ether, ethyl acetate and a 20:1 ethyl acetate/diethyl amine mixture. From the ethyl acetate/diethyl amine fractions the mixture of pyrimidinophanes **3a** and **3b** was obtained as oil in a yield of 1.2 g (17%); R_f 0.48 and 0.54 (6:6:1 ethyl acetate/ethyl ether/diethyl amine as the eluent; ^{1}H NMR ($CDCl_3$) δ 1.01 (m, 6H, CH_3), 1.35–1.63 (м, 32H, CH_2), 2.24 (s, 6H, $C_{6(pyr)}CH_3$), 2.38–2.51 (m, 12H, NCH_2), 3.79 (м, 4H, $N_{(пир)}CH_2$), 3.91 (м, 4H, $2N_{(пир)}CH_2$), 5.56 (s, 2H, $C_{5(pyr)}H$); EIMS *m/z* (%rel. int.): 671 (21) $[M + 1]^+$, 670 (50) $[M]^+$, 656 (15), 655 (31), 642 (41), 641 (100), 628 (11), 627 (24), 348 (21), 335 (28), 266 (20), 203 (59), 127 (18); HRMS: calcd for $C_{38}H_{66}N_6O_4$: M = 670.5146, found: *m/z* 670.515 $[M]^+$.

5.1.3. General procedure for obtaining acyclic compounds ***18a*** *and* ***18b***

A mixture of corresponding ω-bromoalkyl-3,6-dimethyluracil **16a** or **16b** (10 mmol) and ω-ethylaminoalkyl-3,6-dimethyluracil **17a** or **17b** (10 mmol) in the presence of K_2CO_3 (50 mmol) in acetonitrile (100 ml) was refluxed for 12 h. The mixture was evaporated in a vacuum, and the residue was treated with chloroform to extract the reaction products. The precipitate that formed was filtered off, the filtrate was concentrated to 10–20 ml and submitted to chromatography over Al_2O_3. The column was washed with ethyl acetate in the case of **18a** and with chloroform in the case of **18b** to give desired compounds.

*5.1.3.1. Bis[4-(3,6-dimethyluracil-1-yl)butyl]ethylamine (**18a**).* Yield 23%; m.p. 88 °C; R_f 0.27 (10:1 ethyl acetate/diethyl amine as the eluent); ^{1}H NMR ($CDCl_3$): δ 1.03 (t, $J = 7.0$ Hz, 3H, CH_3); 1.66–1.53 (m, 8H, CH_2), 2.26 (s, 6H, $C_{6(pyr)}CH_3$), 2.48 (m, 6H, NCH_2), 3.32 (s, 6H, $N_{(pyr)}CH_3$), 3.83 (t, $J = 7.3$ Hz, 4H, $N_{(pyr)}CH_2$), 5.60 (2H, s, $C_{5(pyr)}H$); Anal. calcd for $C_{22}H_{35}N_5O_4$: C, 60.95; H, 8.14; N, 16.15; found: C, 61.10; H, 8.03; N, 16.13.

*5.1.3.2. Bis[5-(3,6-dimethyluracil-1-yl)pentyl]ethylamine (**18b**)*. Yield 81% as oil; R_f 0.32 (10:10:1 ethyl acetate/ethyl ether/diethyl amine 10:10:1 as the eluent); ^{1}H-NMR ($CDCl_3$): δ 1.00 (t, J = 7.2 Hz, 3H, CH_3); 1.35 (m, 4H, CH_2), 1.47 (m, 4H, CH_2), 1.66 (m, 4H, CH_2), 2.25 (s, 6H, $C_{6(pyr)}CH_3$), 2.41 (t, J = 7.5 Hz, 4H, NCH_2), 2.50 (q, J = 7.2, 2H, NCH_2,), 3.32 (s, 6H, $N_{(pyr)}CH_3$), 3.80 (t, J = 7.9 Hz, 4H, $N_{(pyr)}CH_2$); 5.60 (s, 2H, $C_{5(pyr)}H$); Anal. calcd for $C_{24}H_{39}N_5O_4$: C, 62.45; H, 8.52; N, 15.17; found: C, 62.40; H, 8.43; N, 15.19.

*5.1.4. Synthesis of pyrimidinocyclophane (**21**)*

*5.1.4.1. 1,3-Bis(5-bromopentyl)thymine (**20**)*. A solution of 1,5-dibromopentane (155.9 g, 677.8 mmol) in DMF (90 ml) was added dropwise with stirring to a suspension of 14.4 g (84.7 mmol) of disodium salt of thymine in DMF (150 ml). The mixture was stirred for 5 h at 50–60 °C, after which it was evaporated in a vacuum and the residue was treated with 150 ml of $CHCl_3$. The precipitate that formed was filtered off. The solution was concentrated and submitted to chromatography over Al_2O_3. The column was successively washed with petroleum ether and a 2:1 ether/petroleum ether mixture. From ether/petroleum ether mixture fractions compound **6** was obtained as oil in a yield of 15.9 g (45%); ^{1}H NMR ($CDCl_3$): δ 1.50 (m, 4H, CH_2), 1.76–1.58 (m, 4H, CH_2), 1.89 (m, 4H, CH_2), 1.93 (c, 3H, $C_{5(pyr)}CH_3$), 3.42 (м, 4H, CH_2Br), 3.73 (t, J = 7 Hz, 2H, $N_{(pyr)}CH_2$), 3.95 (t, J = 7 Hz, 2H, $N_{(pyr)}CH_2$), 7.01 (s, 1H, $C_{6(pyr)}H$); EIMS (EI) *m/z* (%rel. int.): 426 (9) $[M]^+$, 424 (22) $[M]^+$, 422 (10) $[M]^+$, 346 (28), 345 (88), 344 (28), 343 (88), 275 (75), 195 (86), 140 (100); Anal. Calcd. for $C_{15}H_{24}Br_2N_2O_2$: C, 42.47; H, 5.70; N, 6.60; Br, 37.68. Found: C, 42.48; H, 5.81; N, 6.53; Br, 37.75.

*5.1.4.2. 15-Methyl-7-butyl-1,7,13-triazabicyclo[11.3.1]heptadeca-15-en-14,17-dione (**21**)*. At 70 °C to a stirred mixture of *n*-butylamine (2.07 g, 28.4 mmol), K_2CO_3 (4.00 g, 29.0 mmol) and catalytic amount TBA · HSO_4 in *n*-BuOH (250 ml) compound **20** (3.0 g, 7.08 mmol) in *n*-BuOH solution (100 ml)was added and stirring was continued for 11.5 h at 70–75 °C. After evaporating of the solvent, treating by $CHCl_3$, filtering and concentration of $CHCl_3$ solution residue was eluated through column with Al_2O_3 by 2:1 ether/petroleum ether mixture. From the fractions of the eluent pyrimidinocyclophane **21** was obtained in a yield of 0.45 g (19%); m.p. 44–45 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 0.85 (t, J = 7.4 Hz, 3H, CH_3), 1.12 (m, J = 7 Hz, 2H), 1.15–1.28 (m, 9H, CH_2), 1.34(m, J = 7.4 Hz, 2H, CH_2), 1.42 (m, 1H, CH), 1.57 (m, 1H, CH), 1.75 (m, 1H, CH), 1.86 (s, 3H, $C_{5(pyr)}CH_3$), 2.11–2.25 (m, 4H, NCH_2), 2.32 (m, 2H, NCH_2), 3.09 (m, 1H, $N_{(pyr)}CH$), 3.92 (m, 1H, $N_{(pyr)}CH$), 4.21 (m, 1H, $N_{(pyr)}CH$), 4.42 (m, 1H, $N_{(pyr)}CH$), 6.83 (s, 1H, $C_{6(pyr)}H$); EIMS *m/z* (%rel. int.): 335 (13) $[M]^+$, 292 (100) $[M - 43]^+$, 278 (8) $[M - 57]^+$; HRMS: calcd for $C_{19}H_{33}N_3O_2$: M = 335.2573, found *m/z* 335.257 $[M]^+$; calcd for $C_{16}H_{26}N_3O_2$: M = 292.2025, found *m/z* 292.202 $[M - 43]^+$.

*5.1.5. General procedure for the quaternization of pyrimidinophanes **1a**, **1b**, **2b**, **3a** and **3b**, **21**, acyclic compounds **18a**, **18b** and **19***

A solution of macrocyclic or acyclic compound (0.30 mmol) and 2–4-fold excess of *n*-decyl bromide or 1.2-fold excess of benzyl bromide in acetonitrile (30 ml) was refluxed for 20 h. The solvent was distilled off. The residue was thoroughly triturated in ethyl ether (5 × 30 ml), each time decantated and finally the solvent was evaporated.

*5.1.5.1. 14,26-Dimethyl-6,20-diethyl-6,20-dibenzyl-1,6,11,15,20,25-hexaazatricyclo-[23,3,1,$1^{11,15}$]-triaconta-13,26-diene-12,28,29,30-tetraone dibromide (**7a**)*. Yield 75%; m.p. 100–110 °C (dec.); ^{1}H NMR (CD_3CN).

δ 1.39 (м, 6H, CH_3), 1.66–1.77 (м, 16H, CH_2), 2.34 (c, 6H, $C_{6(pyr)}CH_3$), 3.19 (м, 12H, NCH_2), 3.88 (м, 8H, $N_{(pyr)}CH_2$), 4.42 (br.s, 2H, CH_2Ph), 4.50 (br.s, 2H, CH_2Ph), 5.59 (c, 2H, $C_{5(pyr)}H$), 7.52 (м, 10H, Ar-H); Anal. calcd for $C_{44}H_{64}Br_2N_6O_4$: C, 58.67; H, 7.16; Br 17.74; N, 9.33; found: C, 58.62; H, 7.10; Br 17.82; N, 9.41.

*5.1.5.2. 14,28-Dimethyl-6,20-diethyl-6,20-dibenzyl-1,6,11,15,20,25-hexaazatricyclo-[23,3,1,$1^{11,15}$]-triaconta-13,27-diene-12,26,29,30-tetraone dibromide (**7b**)*. Yield 62%; m.p. 100–110 °C (dec.); ^{1}H NMR (CD_3CN).

δ 1.33 (м, 6H, CH_3), 1.66–1.77 (м, 16H, CH_2), 2.25 (c, 6H, $C_{6(pyr)}CH_3$), 3.13 (м, 12H, NCH_2), 3.83 (м, 8H, $N_{(pyr)}CH_2$), 4.40 (br. m, 4H, CH_2Ph,), 5.52 (c, 2H, $C_{5(pyr)}H$), 7.53 (м, 10H, Ar-H); Anal. calcd for $C_{44}H_{64}Br_2N_6O_4$: C, 58.67; H, 7.16; Br 17.74; N, 9.33; found: C, 58.60; H, 7.14; Br 17.77; N, 9.39.

*5.1.5.3. 16,30-Dimethyl-7,23-diethy-7,23-di-n-decyl-1,7,13,17,23,29-hexaazatricyclo-[27,3,1,$1^{13,17}$]-tetratriaconta-15,30-diene-14,32,33,34-tetraone dibromide (**9**)*. Yield 90%; m.p. 75–100 °C (dec.); ^{1}H NMR (CD_3CN).

δ 1.24–1.70 (m, 68H, CH_2, CH_3), 2.25 (s, 6H, $C_{6(pyr)}CH_3$), 3.07–3.23 (m, 16H, NCH_2), 3.76–3.86 (m, 8H, $N_{(pyr)}CH_2$), 5.50 (s, 2H, $C_{5(pyr)}H$). Anal. calcd for $C_{54}H_{100}Br_2N_6O_4$: C, 61.35; H, 9.53; Br 15.12; N, 7.95; found: C, 61.12; H, 9.36; Br 15.34; N, 7.86; Anal. calcd for $C_{54}H_{100}Br_2N_6O_4$: C, 61.35; H, 9.53; Br 15.12; N, 7.95; found: C, 61.33; H, 9.54; Br 15.02; N, 8.07.

*5.1.5.4. 18,34-Dimethyl-8,26-diethyl-8,26-dibenzyl-1,8,15,19,26,33-hexaazatricyclo-[31,3,1,$1^{15,19}$]-octatriaconta-17,34-diene-16,36,37,38-tetraone dibromide (**10a**) and 18,36-dimethyl-8,26-diethyl-8,26-dibenzyl-11,8,15,19,26,33-hexaazatricyclo-[31,3,1,$1^{15,19}$]-octatriaconta-17,35-diene-16,34,37,38-tetraone dibromide (**10b**)*. Yield 77%; m.p. > 70 °C (dec.); ^{1}H NMR (CD_3CN).

δ 1.79–1.37 (м, 38H, CH_2, CH_3), 2.28 (c, 6H, $C_{6(pyr)}CH_3$), 3.09–3.34 (м, 12H, CH_2), 3.80(м, 8H, $N_{(pyr)}CH_2$), 4.50 (м, 4H, CH_2Ph), 5.62 (s, 2H, $C_{5(pyr)}H$), 7.52 (м, 10H, Ar-H); Anal.

calcd for $C_{52}H_{80}Br_2N_6O_4$: C, 61.65; H, 7.96; Br 15.78; N, 8.30; found: C, 61.73; H, 8.04; Br 15.66; N, 8.27.

*5.1.5.5. 18,34-Dimethyl-8,26-diethyl-8,26-di-n-decyl-1,8,15,19,26,33-hexaazatricyclo-[31,3,1,1^{15,19}]-octatriaconta-17,34-diene-16,36,37,38-tetraone dibromide (**11a**) and 18,36-dimethyl-8,26-diethyl-8,26-di-n-decyl-11,8,15,19,26,33-hexaazatricyclo-[31,3,1,1^{15,19}]-octatriaconta-17,35-diene-16,34,37,38-tetraone dibromide (**11b**)*. Yield 71%; m.p. 60–75 °C (dec.); ^{1}H NMR (CD_3CN).

δ 1.29–1.82 (m, 76H, CH_2, CH_3), 2.23 (s, 6H, $C_{6(pyr)}CH_3$), 3.08–3.20 (m, 16H, NCH_2), 3.81–3.87 (m, 8H, $N_{(pyr)}CH_2$), 5.51 (s, 2H, $C_{5(pyr)}H$); Anal. calcd for $C_{58}H_{108}Br_2N_6O_4$: C, 62.57; H, 9.78; Br 14.35; N, 7.55; found: C, 62.68; H, 9.87; Br 14.24; N, 7.49.

*5.1.5.6. Bis[4-(3,6-dimethyluracil-1-yl)butyl]benzylethylammonium bromide (**12**)*. Yield 71%; m.p. > 110 °C (dec.); ^{1}H NMR ($CDCl_3$).

δ 1.38 (t, J = 7.3 Hz, 3H, CH_3), 1.64 (m, 4H, CH_2), 1.82 (m, 4H, CH_2), 2.26 (s, 6H, $C_{6(pyr)}CH_3$), 3.16–3.24 (m, 6H, NCH_2), 3.19 (s, 6H, $N_{(pyr)}CH_3$); 3.86 (t, J = 7.6 Hz, 4H, $N_{(pyr)}CH_2$,), 4.53 (s, 2H, CH_2Ph,), 5.55 (2H, s, $C_{5(pyr)}H$), 7.42 (м, 5H, Ar-H); Anal. calcd for $C_{29}H_{42}BrN_5O_4$: C, 57.61; H, 7.00; Br, 13.22; N, 11.58; found: C, 57.73; H, 7.09; Br, 13.34; N, 11.51.

*5.1.5.7. Bis[5-(3,6-dimethyluracil-1-yl)pentyl]-n-decylethylammonium bromide (**13**)*. Yield 79%; m.p. > 70 °C (dec.); ^{1}H-NMR ($CDCl_3$).

δ 0.88 (t, J = 6.9 Hz, 3H, CH_3), 1.29 (m, 19H, CH_3, CH_2); 1.66 (m, 12H, CH_2), 2.25 (s, 6H, $C_{6(pyr)}CH_3$), 3.06 (m, 8H, NCH_2,), 3.19 (s, 6H, $N_{(pyr)}CH_3$), 3.81 (t, J = 7.6 Hz, 4H, $N_{(pyr)}CH_2$,); 5.54 (s, 2H, $C_{5(pyr)}H$); Anal. calcd for $C_{34}H_{60}BrN_5O_4$: C, 59.81; H, 8.86; Br, 11.70; N, 10.26; found: C, 59.90; H, 8.85; Br, 11.74; N, 10.31.

*5.1.5.8. 1,3-Bis[5-(n-decyldiethylammonium)pentyl]-6-methyluracil dibromide (**14**)*. Yield 52%; m.p. > 80 °C (dec.); ^{1}H NMR ($CDCl_3$).

δ 0.88 (m, 6H, CH_3), 1.26 (m, 12H, CH_3), 1.38–1.52 (m, 32H, CH_2), 1.71–1.92 (m, 12H, CH_2), 2.34 (s, 6H, $C_{6(pyr)}CH_3$), 3.28–3.32 (m, 8H, NCH_2), 3.49–3.53 (m, 8H, NCH_2), 3.90 (м, 4H, $N_{(пир)}CH_2$), 5.55 (s, 1H, $C_{5(pyr)}H$): Anal. calcd for $C_{43}H_{86}Br_2N_4O_2$: C, 60.69; H, 10.19; Br 18.78; N, 6.58; found: C, 60.79; H, 10.01; Br 18.82; N, 6.55.

*5.1.5.9. 15-Methyl-7-butyl-7-n-decyl-1,7,13-triazabicyclo [11.3.1]heptadeca-15-en-14,17-dione bromide (**22**)*. Yield 33%; m.p. > 100 °C (dec.); ^{1}H NMR ($CDCl_3$).

δ 0.95 (m, 6H, CH_3), 1.34–1.85 (m, 32H, CH_2), 1.97 (s, 3H, $C_{5(pyr)}CH_3$), 2.88–3.01 (m, 6H, NCH_2), 3.72–4.10 (m, 6H, $N_{(пир)}CH_2$, NCH_2), 7.00 (s, 1H, $C_{6(pyr)}H$); Anal. calcd for $C_{29}H_{54}BrN_3O_2$: C, 62.57; H, 9.78; Br, 14.35; N, 7.55; found: C, 62.44; H, 9.71; Br, 14.23; N, 7.62.

5.2. Biological evaluation

5.2.1. Antibacterial and antifungal activity

The in vitro antibacterial and antifungal activity of the macrocyclic and acyclic synthesized compounds were investigated against several pathogenic representative Gram-negative bacteria (*Pseudomonas aeruginosa* 9027, *Escherichia coli* F-50), Gram-positive bacteria (*Staphylococcus aureus* 209p, *Bacillus subtilis* 6633, *Enterococcus faecalis* ATCC 8043), moulds (*Aspergillus niger* BKMF-1119, *Aspergillus fumigatus* AF-27, *Trichophyton mentagrophytes var. gypseum* 1773) and yeast (*Candida Albicans* 885–653). Minimal inhibitory concentrations (MICs) were estimated by conventional dilution methods for bacteria [23] and fungi [24]. 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). Ampicillin, ciprofloxacin, amphotericin B and clotrimazole 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 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 activity was determined as follows. Assay tubes were filled with 1 ml of test compound solution in nutrient agar. Concentrations of test compounds were varied from 12.5 to 10^4 μg/ml. Normal saline broth (bacteria 3×10^5 cfu/ml), 1 ml was added to the tubes and for 5 min, 10 min, 15 min, 30 min, 1 h, 1.5 h, 2 h, 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 total cell death. For fungicidal activity determination the tubes with the test compounds and fungi were incubated at 26 °C. For 10 min, 20 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 6 h the inocula were prepared in Sabouraud dextrose broth and incubated at 26 °C.

5.2.2. Toxicity

Toxicity tests were carried out via: (1) Single peroral (per os) introductions of pyrimidinophanes aqueous solutions in acute tests on white outbreed mice of both sexes with the mass of 17–21 g. The observation period was 72 hours. As the criteria of toxicity, the average lethal doses were used—LD_{50}. To measure these values each compound was introduced to five groups of mice (10 mice per dose; N = 50); (2) Using laboratory partenogenetic culture of *Daphnia magna Straus* (water solutions of compounds were used) at the age of 18 ± 6 hours, kept in standard conditions. Sensitivity of daphnia culture was normal, i.e. 0.9–2.0 mg/l, as estimated via $LC_{50}^{24\ h}$ of potassium bichromate. Observation period was equal 48 hours. As the criteria of toxicity, the average lethal concentrations were used—$LC_{50}^{48\ h}$. To determine $LC_{50}^{48\ h.}$ each compound was applied for three groups of daphnia (30 daphnia per each concentration; N = 90). The results were processed using the program ToxCalc™ v.5.0.23E (Tidepool Scientific Software; USA) [21].

References

[1] Y.S. Shvetsov, I.S. Salikhov, V.S. Reznik, Izv. Akad. Nauk SSSR, Ser. Khim. 25 (1976) 1103–1106 [Bull. Acad. Sci. USSR, Div.Chem. Sci. (Engl. Transl.). 25 (1976)].
[2] M.M. Htay, O. Meth-Cohn, Tetrahedron Lett. (1976) 79–82.
[3] K. Golankiewicz, B. Skalski, J. Polish, Chem. 52 (1978) 1365–1373.
[4] G.R. Newkome, A. Nayak, M.G. Sorci, W.H. Benton, J. Org. Chem. 44 (1979) 3812–3816.
[5] B. Muke, T. Kauffmann, Chem. Ber. 113 (1980) 2739–2748.
[6] U. Eiermann, C. Krieger, F.A. Neugebauer, Chem. Ber. 123 (1990) 1885–1889.
[7] T. Itahara, Bull. Chem. Soc. Jpn. 69 (1996) 3239–3246.
[8] V. Caplar, L. Tumir, M. Zinic, Croat. Chem. Acta 69 (1996) 1613–1617.
[9] S. Kumar, G. Hundal, D. Paul, M.S. Hundal, H. Singh, J. Chem. Soc. Perkin Trans. 1 (2000) 2295–2301.
[10] V.E. Semenov, V.D. Akamsin, V.S. Reznik, Y.Y. Efremov, D.R. Sharafutdinova, A.A. Nafikova, et al., Mendeleev Commun (2001) 97–98.
[11] V.E. Semenov, V.D. Akamsin, V.S. Reznik, A.V. Chernova, G.M. Dorozhkina, Y.Y. Efremov, et al., Tetrahedron Lett. 43 (2002) 9683–9686.
[12] A.S. Mikhailov, R.K. Giniyatullin, V.E. Semenov, V.S. Reznik, A.A. Nafikova, S.K. Latypov, Y.Y. Efremov, D.R. Sharafutdinova, et al., Russ. Chem. Bull. Int. Ed. 52 (2003) 1399–1402.
[13] R.K. Giniyatullin, A.S. Mikhailov, V.E. Semenov, V.D. Akamsin, V.S. Reznik, A.V. Chernova, et al., Russ. Chem. Bull. Int. Ed. 52 (2003) 1595–1599.
[14] V.S. Reznik, N.G. Pashkurov, A.S. Mikhailov, I.V. Galyametdinova, V.V. Zobov, A.V. Lantsova, Khim-Pharm. Zh. 38 (2004) 22–25 [Pharm. Chem. J. (Engl. Transl.) 38 (2004)].
[15] V.S. Reznik, K.A. Anikienko, V.K. Kurochkin, V.D. Akamsin, I.V. Galyametdinova, E.A. Bychikhin, Doklady Chemistry. (Engl. Transl.) 362 (1998) 68–70.
[16] K.A. Anikienko, E.A. Bychikhin, V.K. Kurochkin, V.S. Reznik, V.D. Akamsin, I.V. Galyametdinova, Doklady Chemistry. (Engl. Transl.) 376 (2001) 39–43.
[17] O.V. Gorshkova, V.V. Zobov, E.A. Bukharaeva, E.E. Nikol'skij, V.D. Akamsin, I.V. Galyametdinova, et al., Bull. Exp. Biol. Med. (Engl. Transl.) 131 (2001) 446–450.
[18] N.S. Karamova, O.B. Ivanchenko, O.N. Ilinskaya, V.V. Zobov, V.S. Reznik, Arch. Toxicol. 76 (2002) 122–126.
[19] I.V. Kovyazina, K.A. Petrov, V.V. Zobov, E.A. Bukharaeva, E.E. Nikolsky, Doklady Chemistry. (Engl. Transl.) 399 (2004) 458–460.
[20] V.V. Zobov, K.A. Petrov, A.A. Aslyamova, L.A. Berezinskii, V.D. Akamsin, I.V. Galyametdinova, et al., Doklady Chemistry. (Engl. Transl.) 401 (2005) 77–80.
[21] H.L. Brooks, Pesticide background statements, Volume IV, Intecticides, Department of agriculture, Agriculture handbook, No 685, Forest service, U.S, 1989.
[22] J.C. Kellet, W.C. Doggett Jr., J. Pharm. Sci. 55 (1966) 414–417.
[23] National Committee for Clinical Laboratory Standards, Methods for dilution antimicrobial susceptibility. Tests for bacteria that grow aerobically —sixth edition: approved standard, M7-A5, NCCLS, Wayne, Pa., USA, 2000.
[24] National Committee for Clinical Laboratory Standards, Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi: proposed standard, M38-P, NCCLS, Wayne, Pa., USA, 1998.