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In vitro antibacterial activity of ten series of substituted quinazolines

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The antibacterial activity of ten series of substituted quinazolines (157 derivatives) against bacterial strains *Escherichia coli* CCM 3988, *Pseudomonas aeruginosa* CCM 3955, *Bacillus subtilis* ATCC 6663 and *Staphylococcus aureus* CCM 3953 by microdilution assay was investigated. The sensitivity of the Gram positive bacteria to the tested quinazolines was higher than that of Gram negative bacteria. The most effective of ten quinazoline structure series were condensed [1,2,4]triazoloquinazolines and 10*H*-[1,2,4]triazino[5,4-*b*]quinazolin-10-ones. Study of structure-activity relationship showed that the most effective derivatives were those carrying a unsubstituted benzene ring or one substituted with small substituents (Cl and CH_3) while having pyrimidine ring substituted with larger substituents, such as morpholine, phenyl or secondary amines. The most effective derivative 1-[(3-methylphenyl)amino]-10*H*-[1,2,4]triazino[5,4-*b*]quinazolin-10-one had MIC values 5 mg/L for *E. coli*; 100 mg/L for *P. aeruginosa*, 10 mg/L for *S. aureus* and 1 mg/L for *B. subtilis*. 9-chloro-morpholin-4-yl [1,2,4]triazolo[4,3-*c*]quinazolin-3(4*H*)-thione demonstrated MIC value lower than ampicillin for *B. subtilis* and the same MIC value as ampicillin for *E. coli*.

Key words: quinazolines, antibacterial activity, structure-activity relationship.

Introduction

The ever-growing resistance to antibiotics leads to continuous screening for new biologically effective substances of natural or synthetic origin (Aguilar et al., 2002).

Quinazolines, the derivatives of benzopyrimidine, are the compounds used in the pharmaceutical industry, in medicine and in agricultures because of their wide spectrum of biological activity. The connection between a wide spectrum of biological activities and compounds containing the quinazoline moiety has been known and is well documented in the literature. Such compounds have been known as antimalarials, diuretics. For example, the biological effects of some 50 quinazo-

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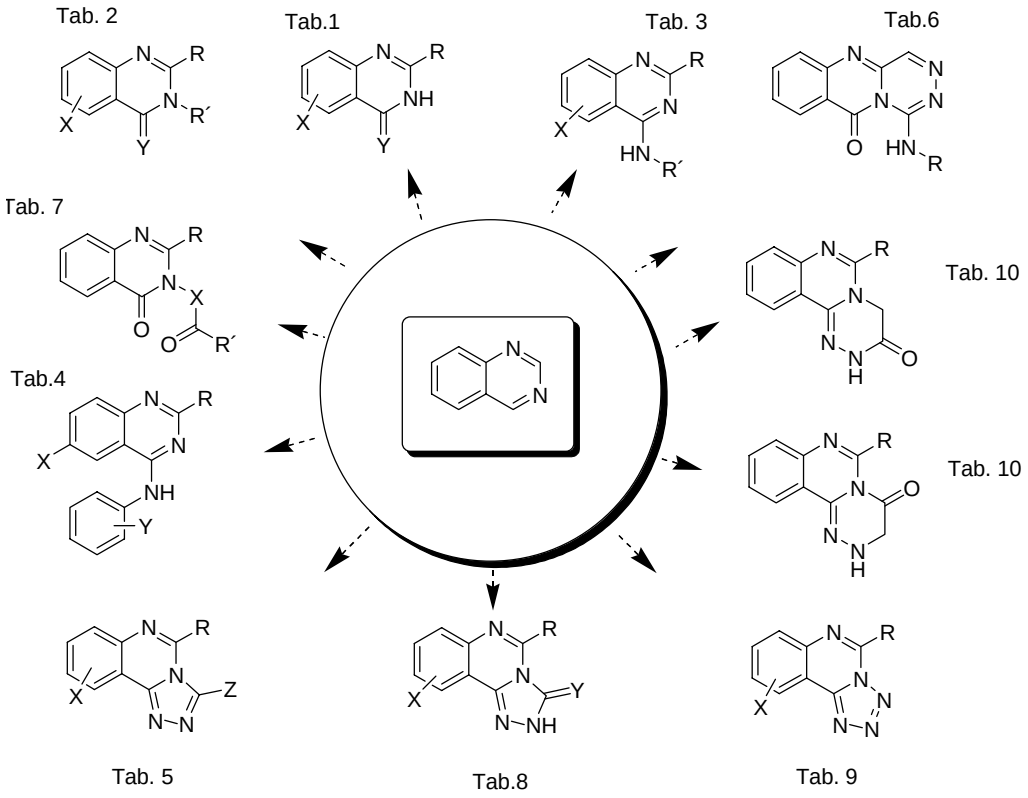


Figure 1. Survey of compounds studied in this work.

line alkaloids occurring in nature were described in the Armarego monographs (Armarego, 1967, 1979). Up to 1980 several review articles, devoted to biological phytoeffects and pharmacological research of quinazolines, were written (Jachontov et al., 1977; Johne, 1981; Suesse & Johne, 1981). Due to an unprecedented interest in pharmacologically utilizable quinazolines, in the last two decades more than one thousand quinazolines, quinazolones, their thioanalogues and condensed quinazolines have been synthesized.

In our search for antibacterial agents, we have focused on synthesis and developing of quinazoline derivatives, which may inhibit bacterial growth (Bodajla et al., 1994; Jantová et al., 1994a,b, 1995, 1999, 2000). Our studies, investigating the relations between the structure of quinazolines and their cytotoxic effect on HeLa cells (Jantová et al., 1997), have shown that during the experiment some structural types of the tested quinazolines are considerably metabolized. The speed of the process does not depend on the lipophilicity of the various substances but on their structure.

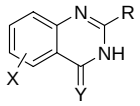
In this work we summarized the antibacterial activity of 157 antibacterial compounds containing the quinazoline skeleton (Fig. 1), prepared in our laboratories. We studied both quinazolines and quinazolin-4-ones, as well as their sulfur analogs, substituted in positions 2, 3 and 4 of the pyrimidine ring and at the benzene ring of quinazoline by typical pharmacophores, such as halogens, nitro and amino containing groups and their derivatives, e.g. arylhydrazones and arylthiosemicarbazones. In addition to substituted quinazolines, we investigated also linearly and angularly fused quinazolines, such as for instance derivatives of 1,2,4-triazolo[4,3-*c*]quinazolines, 3,4-dihydro-2*H*-[1,2,4]triazino[4,3-*c*]quinazolones and 10*H*-[1,2,4]triazino-[5,4-*b*]quinazolines. Further -more we investigated the relationship between the antibacterial effect and the structure of quinazoline derivative tested.

Material and methods

Bacteria

Escherichia coli CCM 3988, *Pseudomonas aeruginosa* CCM 3955, *Bacillus subtilis* ATCC 6663 and *Staphy-*

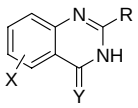
Table 1. Antibacterial effect of 2-substituted quinazoline-4(3H)-ones and their thio analogues. [a]



No.	X	Y	R	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
1	H	O	Phenyl	>100[b]	>100[b]	>100[b]	>100[b]
2	6-Cl	O	Phenyl	>100[b]	>100[b]	100[b]*	>100[b]
3	H	O	H	>100[b]	>100[b]	>100[b]	>100[b]
4	H	S	Phenyl	>100[b]	>100[b]	100[b]*	>100[b]
5	8-CH_3	S	Phenyl	>100[b]	>100[b]	>100[b]	>100[b]
6	6-Br	S	Phenyl	>100[b]	>100[b]	100[b]*	>100[b]
7	6-Cl	S	Phenyl	>100[b]	>100[b]	100[b]*	>100[b]
8	6-Br	S	Morpholin-4-yl	>100[b]	>100[b]	>100[b]	>100[b]
9	6-Cl	S	Morpholin-4-yl	>100[b]	>100[b]	100[b]*	>100[b]
10	6-Cl	S	Piperidin-1-yl	>100[b]	>100[b]	>100[b]	>100[b]
11	6-NO_2	S	Piperidin-1-yl	>100[b]	>100[b]	>100[b]	>100[b]
12	6-Cl	S	4-Phenylpiperazin-1-yl	>100[b]	>100[b]	100[b]*	>100[b]
13	H	S	*N,N*-Diphenylamine	>100[b]	>100[b]	>100[b]	>100[b]
14	H	O	CH_3	>500[c]	>500[c]	>500[c]	>500[c]
15	H	O	CH_2Br	100[c]*	>500[c]	100[c]*	100[c]*
16	H	O	CH_2-morpholin-4-yl	>500[c]	>500[c]	>500[c]	>500[c]
17	H	O	CH_2-piperidin-1-yl	>500[c]	>500[c]	>500[c]	>500[c]
18	H	O	CH_2-methoxy	>500[c]	>500[c]	>500[c]	>500[c]
19	H	O	CH_2-ethoxy	>500[c]	>500[c]	>500[c]	>500[c]
20	H	O	CH_2-isopropoxy	>500[c]	>500[c]	>500[c]	>500[c]
21	H	O	CH_2-butoxy	>500[c]	>500[c]	>500[c]	>500[c]
22	H	O	CH_2-phenoxy	>500[c]	>500[c]	>500[c]	>500[c]
23	H	O	CH_2-4-nitrophenoxy	>500[c]	>500[c]	>500[c]	>500[c]
24	H	O	CH_2-2-nitrophenoxy	>500[c]	>500[c]	>500[c]	>500[c]
25	H	O	CH_2-N,N-dimethylamino	>500[c]	>500[c]	>500[c]	>500[c]
26	H	O	CH2-N-(2-hydroxyethyl)amino	>500[c]	>500[c]	>500[c]	>500[c]
27	H	O	CH2-N-(methoxycarbonyl) methylamino	>500[c]	>500[c]	>500[c]	>500[c]
28	H	O	CH2-N-(1,3-dicarboxypropyl)amino	>500[c]	>500[c]	>500[c]	>500[c]
29	H	O	CH_2-NH-phenyl	>100	>100	>100	>100
30	H	O	CH_2-NH-4-methylphenyl	>100	>100	>100	>100
31	H	O	CH_2-NH-4-trifluoromethylphenyl	>100	>100	>100	>100
32	H	O	CH_2-NH-2-methoxyphenyl	>100	>100	>100	>100
33	H	O	CH_2-NH-2-fluorophenyl	>100	>100	>100	>100
34	H	O	CH_2-NH-4-bromophenyl	>100	>100	>100	>100
35	H	O	CH_2-NH-4-chlorophenyl	>100	>100	>100	>100
36	H	O	CH_2-NH-2,4-dichlorophenyl	>100	>100	10**	>100
37	H	O	CH_2-NH-2,5- dichlorophenyl	>100	>100	>100	>100
38	H	O	CH_2-NH-3,4-dichlorophenyl	>100	>100	>100	>100
39	H	O	CH_2-NH-2,3,4-trichlorophenyl	>100	>100	>100	>100
40	H	O	CH_2-NH-2-carboxyphenyl	>100	>100	>100	>100
41	H	O	CH_2-NH-4-carboxyphenyl	>100	>100	>100	>100
42	H	O	CH2-NH-NH-CS-NH-phenyl	>100	>100	>100	>100
43	H	O	CH2-NH-NH-CS-NH-chlorophenyl	>100	>100	>100	>100
44	H	O	CH2-NH-NH-CS-NH-nitrophenyl	>100	>100	>100	>100
45	H	O	CH2-NH-NH-CS-NH-hydroxyphenyl	>100	>100	>100	>100
46	H	O	CH2-NH-NH-CS-NH-methylphenyl	>100	>100	>100	>100
47	H	O	CH2-NH-NH-CS-NH-izopropyl	>100	>100	>100	>100
Amp				1**	>100	0.04**	10**

[a] Values of MIC are in mg/L; Amp-ampicillin; ** bactericidal effect, * bacteriostatic effect.
[b] Reported in Jantová et al. (2000). [c] Reported in Špirková et al. (1999).

Table 2. Antibacterial activity of 2,3-disubstituted quinazoline-4(3*H*)-ones, resp. thiones. [a]



No.	Y	R	R'	*E. coli*[b]	*P. aeruginosa*[b]	*S. aureus*[b]	*B. subtilis*[b]
48	O	H	COOEt	>100	>100	>100	>100
49	O	Methyl	COOMe	>100	>100	>100	>100
50	O	Phenyl	CH_2COOEt	>100	>100	>100	>100
51	O	H	COOEt	>100	>100	>100	>100
52	O	Methyl	$COCH_2Cl$	>100	>100	>100	50*
53	O	Phenyl	COOMe	>100	>100	>100	>100
54	S	H	–	>100	>100	>100	>100
55	S	Phenyl	–	>100	>100	>100	50*
56	S	Methyl	–	>100	>100	>100	>100
Amp				1**	>100	0.04**	10**

[a] Values of MIC are in mg/L; Amp-ampicillin; ** bactericidal effect, * bacteriostatic effect.
[b] Reported in Jantová et al. (1999).

lococcus aureus CCM 3953 were used. All the strains were obtained from the Czech Collection of Microorganisms (Brno, Czech Republic).

Compounds
The preparation of compounds 1–157 has been described by Stankovský et al. (1980, 1989, 1991) and Špirková et al. (1990, 1993, 1994, 1997, 1999). The compounds were used at concentrations of 100, 50, 25, 10, 5, 1 and 0.1 mg/L for compounds 1–13, 29–56, 96–138 and 156–157; 500, 200, 100, 50, 10, 1 and 0.1 for compounds 14–28, 76–95 and 139–146; and 1000, 500, 100, 50, 10, 5, 1 and 0.1 mg/L for compounds 57–75 and 147–155. Chromatographically purified derivatives were dissolved in dimethyl sulfoxide whose final concentration never exceeded 1% (*V*/*V*) in either control or treated samples. Because the solubility of the studied quinazolines covered a wide range, they were tested in various concentration ranges.

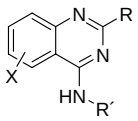
Detection of antibacterial activity
The antibacterial effect was assayed by a microdilution method in 96-well microtitration plates (Jantová et al., 1995). The bacteria were cultured on Müller-Hinton medium at 30 °C. An overnight inoculum was prepared 12–16 h before the test. The growing inoculum was filtered and a 1.5% suspension of bacteria was prepared for the experiments. This suspension (180 μL) was added to 20 μL of the tested complex solution and cultured for 8 h on a reciprocal shaker in a thermostat at 30 °C. The time course of absorbance (A_{630}) was then determined in three parallels. To compare the antibacterial activity, ampicillin at concentrations 100, 10, 1, 0.1 and 0.01 mg/L was used as standard. The antibacterial effect was characterized by IC_{50} values, *i.e.* the minimal inhibition concentration of a substance which inhibits bacterial growth by 50% relative to the control, and MIC, *i.e.* the minimal inhibitory concentration of a substance which inhibits the bacterial growth by 100%. The IC_{50} and MIC values were determined from toxicity curves.

Results

The results obtained in the antibacterial tests are summarized in Tables 1–10. Table 1 represents the antibacterial effect of the substituted quinazoline-4(3*H*)-ones and their thioanalogues. The sensitivity of Gram positive bacteria to the above-mentioned derivatives was higher than that of Gram negative bacteria. The bacterium *S. aureus* was the higher sensitive to substituted quinazoline-4(3*H*)-ones and their thioanalogues. MIC values show that the antibacterial effect was found with derivatives 2, 4, 6, 7, 9, 12, 15 and 36. Derivatives 2, 4, 6, 7, 9, 12 and 36 were active against *S. aureus*. Only derivative 15 was effective against Gram positive *S. aureus*, *B. subtilis* and Gram negative *E. coli*. A concentration 100 mg/L of quinazoline 15 caused bacteriostatic effect. A concentration 10 mg/L of quinazoline 36 exerted a bactericidal effect on *S. aureus*. The other substituted quinazoline-4(3*H*)-ones and their thioanalogues tested were inactive.

Table 2 shows the MIC values of nine 2,3-disubstituted quinazoline-4(3*H*)-ones and thiones. Only the bacterium *B. subtilis* was sensitive to the effects of two quinazolines. A concentration 50 mg/L of quinazolines 52, 55 induced bacteriostatic effect on *B. subtilis*. No antibacterial activity was found with other 2,3-disubstituted quinazoline-4(3*H*)-ones and thiones.

Table 3. Antibacterial activity of (a) 4-quinazolylhydrazines and their arylhydrazones and (b) 4-quinazolylthiosemicarazides. [a]



(a)

No.	X	R	R'	*E. coli*[b]	*P. aeruginosa*[b]	*S. aureus*[b]	*E. faecalis*[b]
57	6-Cl	Morpholin-4-yl	NH_2	>1000	>1000	>1000	>1000
58	6-Cl	Piperidin-1-yl	NH_2	>1000	500**	>1000	500*
59	6-Cl	Phenyl	NH_2	500**	>1000	500**	250**
60	6-Br	Morpholin-4-yl	NH_2	500*	>1000	250**	>1000
61	H	Piperidin-1-yl	NH_2	>1000	>1000	250**	>1000
62	H	Phenyl	NH_2	>1000	1000*	500**	>1000
63	8-CH_3	Morpholin-4-yl	NH_2	1000*	>1000	1000*	500*
64	8-CH_3	Phenyl	NH_2	1000*	250*	94*	1000*
65	6-Cl	Morpholin-4-yl	N=CH-2-furyl	>1000	>1000	>1000	>1000
66	6-Cl	Morpholin-4-yl	N=CH-4-nitrophenyl	>1000	>1000	>1000	>1000
67	6-Cl	Morpholin-4-yl	N=CH-2-nitrophenyl	>1000	>1000	>1000	>1000
68	6-Cl	Morpholin-4-yl	N=CH-2-chlorophenyl	>1000	>1000	>1000	>1000
69	6-Cl	Morpholin-4-yl	N=CH-4-acetamidophenyl	>1000	>1000	>1000	>1000
70	6-Cl	Morpholin-4-yl	N=CH-5-bromo-2-furyl	1000*	>1000	>1000	>1000
71	6-Cl	Morpholin-4-yl	N=CH-5-nitro-2-furyl	500*	500*	250**	100**
72	8-CH_3	Phenyl	2-furyl	>1000	>1000	>1000	1000
73	8-CH_3	Phenyl	N=CH-phenyl	1000*	1000*	>1000	>1000
74	8-CH_3	Phenyl	N=CH-4-*N*, *N*-dimethylaminophenyl	500*	500*	500*	500*
75	8-CH_3	Phenyl	N=CH-4-nitrophenyl	>1000	>1000	>1000	>1000
Amp				1**	>500	0,04**	1**

(b)

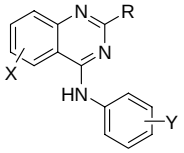
No.	X	R	R'	*E. coli*[c]	*P. aeruginosa*[c]	*S. aureus*[c]	*B. subtilis*[c]
76	6-Cl	Phenyl	NH-CS-NH-phenyl	>200	>200	>200	>200
77	6-Cl	Morpholin-4-yl	NH-CS-NH-phenyl	>200	>200	>200	>200
78	6-Cl	Piperidin-1-yl	NH-CS-NH-phenyl	>200	>200	>200	>200
79	6-Cl	4-*N*-Phenylpiperidin-1yl	NH-CS-NH-phenyl	>200	>200	>200	>200
80	8-CH_3	Phenyl	NH-CS-NH-phenyl	10-100	10-100	10-100	10-100
81	8-CH_3	Phenyl	NH-CS-NH-(4-NO_2-phenyl)	10**	>200	>200	1*
82	6-Cl	*N*,*N*-Diethylamine	NH-CS-NH-phenyl	>200	>200	>200	>200
83	6-Br	Morpholin-4-yl	NH-CS-NH-phenyl	>200	>200	>200	>200
Amp				1**	>500	0.04**	10**

[a] Values of MIC are in mg/L; Amp-ampicillin; ** bactericidal effect, * bacteriostatic effect.
[b] Reported in Jantová et al. (1995).
[c] Reported in Jantová et al. (1994b).

The activity of 4-quinazolylhydrazines, their arylhydrazones and 4-quinazolylthiosemicarbazides is demonstrated in Table 3. The broadest antibacterial effect was found with derivatives 64, 71, 74 and 80 which were effective with all the tested bacteria. No antibacterial activity was found with derivatives 57, 65, 66, 67, 68, 69, 75, 76, 77, 78, 79, 82 and 83. The sensitivity of Gram positive bacteria to the derivatives was higher than that of Gram negative bacteria.

Antibacterial activity of 2,6-disubstituted 4-anilinoquinazolines is demonstrated in Table 4. None of the prepared quinazolines influenced the Gram negative *E. coli* and *P. aeruginosa*. The widest antibacterial spectrum was encountered with 4-anilinoquinazolines 92 and 93 which were

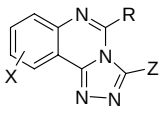
Table 4. Antibacterial activity of 2,6-disubstituted 4-anilinoquinazolines. [a]



No.	X	R	Y	*E. coli*[b]	*P. aeruginosa*[b]	*S. aureus*[b]	*B. subtilis*[b]
84	6-Br	2-Diethylamino	4'-NO_2	>500	>500	>500	10<MIC<100
85	6-Br	2-Piperidin-1-yl	2'-NO_2	>500	>500	>500	>500
86	6-Br	2-Morpholin-4-yl	4'-Br	>500	>500	>500	>500
87	6-Br	2-Morpholin-4-yl	2'-NO_2	>500	>500	>500	>500
88	6-Br	2-Morpholin-4-yl	4'-NO_2	>500	>500	>500	>500
89	6-Br	2-Morpholin-4-yl	H	>500	>500	>500	>500
90	H	Morpholin-4-yl	4'-Br	>500	>500	>500	>500
91	6-Cl	Morpholin-4-yl	4'-NO_2	>500	>500	>500	>500
92	H	Phenyl	H	>500	>500	10**	10<MIC<100
93	H	Phenyl	4'-CH_3	>500	>500	1<MIC<10	0.1<MIC<1**
94	6-CH_3	Phenyl	4'-Cl	>500	>500	>500	>500
95	6-Cl	Phenyl	4'-CH_3	>500	>500	>500	>500
Amp				1**	>500	0.04**	10**

[a] Values of MIC are in mg/L; Amp-ampicillin; ** bactericidal effect, * bacteriostatic effect.
[b] Reported in Jantová et al. (1994a).

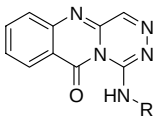
Table. 5 Antibacterial activity of some [1,2,4]triazolo[4,3-*c*]quinazolines. [a]



No.	X	R	Z	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
96	9-Cl	Morpholin-4-yl	NH-phenyl	10–100	10–100	10–100	10–100
97	9-Cl	Piperidin-1-yl	NH-phenyl	10–100	10–100	10–100	10–100
98	9-Cl	4-Phenylpiperazin-1-yl	NH-phenyl	>200	>200	>200	>200
99	7-Me	Phenyl	NH-phenyl	>200	>200	>200	>200
100	9-Cl	Morpholin-4-yl	Indol-3-yl	>100	>100	100**	100**
101	9-Cl	Morpholin-4-yl	5-nitrothiophen-2-yll	>100	>100	50**	10**
102	9-Cl	Morpholin-4-yl	5-nitrofuran-2-yl	>100	>100	50**	10**
103	9-Cl	Morpholin-4-yl	5-phenylsulfonylfuran-2-yl	>100	>100	>100	>100
104	9-Cl	Morpholin-4-yl	2-acetylfuran-2-yl	>100	>100	>100	>100
105	9-Cl	Morpholin-4-yl	5-bromofuran-2-yl	>100	>100	100*	50*
106	9-Cl	Morpholin-4-yl	4-acetamidophenyl	>100	>100	>100	>100
107	9-Cl	Morpholin-4-yl	2-nitrophenyl	>100	>100	>100	>100
108	9-Cl	Morpholin-4-yl	2-chlorophenyl	>100	>100	>100	>100
109	9-Cl	4-Phenylpiperazin-1-yl	4-chlorophenyl	>100	>100	>100	>100
110	9-Cl	4-Phenylpiperazin-1-yl	5-nitrofuran-2-yl	>100	>100	100**	50**
111	9-Cl	4-Phenylpiperazin-1-yl	5-chlorofuran-2-yl	>100	>100	>100	>100
112	9-Cl	4-Phenylpiperazin-1-yl	furan-2-yl	>100	>100	>100	>100
113	9-Cl	4-Phenylpiperazin-1-yl	5-bromofuran-2-yl	>100	>100	>100	>100
114	9-Cl	4-Phenylpiperazin-1-yl	phenyl	>100	>100	>100	>100
115	9-Cl	4-Phenylpiperazin-1-yl	4-*N*,*N*-dimethylaminophenyl	>100	>100	>100	>100
Amp				1**	>500	0.04**	10**

[a] Values of MIC are in mg/L; Amp-ampicillin; ** bactericidal effect.

Table 6. Antibacterial activity of some 1-(alkyl/aryl-amino)-10*H*-[1,2,4]triazino[5,4-b] quinazolin-10-ones. [a]



No.	R	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
116	Phenyl	5*	>100	25*	1**
117	4-chlorophenyl	10*	>100	100*	5*
118	2-nitrophenyl	5*	>100	25*	1*
119	3-hydroxyphenyl	>100	>100	>100	>100
120	3-methylphenyl	5*	100*	10*	1**
121	isopropyl	10**	>100	25*	1**
Amp		1**	>100	0.04**	10**

[a] Values of MIC are in mg/L; Amp-ampicillin; ** bactericidal effect, * bacteriostatic effect.

effective against *B. subtilis* and *S. aureus*. Derivative 84 influenced only Gram positive*B. subtilis*.

Table 5 shows the activity of substituted 1,2,4-triazolo[4,3-*c*]quinazolines. The broadest antibacterial spectrum was demonstrated with derivatives 96 and 97 which were effective with all the tested bacteria. Derivatives 100, 101, 102, 105 and 110 influenced Gram positive *S. aureus* and *B. subtilis*. The MIC values of these derivatives exerted bactericidal effect on Gram positive bacteria. The effect of derivatives 101 and 102 on *B. subtilis* was the same as that of ampicillin. Minimal inhibition concentration (10 mg/L) exhibited bactericidal effect.

The MIC values of the 10*H*-[1,2,4]triazino[5, 4-b]quinazolin-10-ones against Gram positive and Gram negative bacteria is shown in Table 6. Five derivatives (116, 117, 118, 120, 121) were effective on the three bacterial strains tested. The highest activity of these compounds was against *B. subtilis*. A concentration of 1 mg/L of 118 induced 100% inhibition of the bacterial growth. On the other hand, the concentration of derivatives 116, 120 and 121 exhibited bactericidal effect against *B. subtilis*. The MIC values for *B. subtilis* was 2-times (for derivative 117) and 10-times (for derivatives 116, 118, 120 and 121) lower than the corresponding value for ampicillin. Derivative 120 exhibited the highest antibacterial activity on *S. aureus* (MIC = 10 mg/L). A certain antibacterial effect on *S. aureus* was manifested by derivatives 116, 118, and 121, too (MIC = 25 mg/L). Derivatives 116, 118 and 120 demonstrated the highest antibacterial activity on *E. coli* (MIC = 5 mg/L). Minimal inhibition concentration of derivative 121 was 10 mg/L and it was also the concentration which induced bactericidal effect on *E. coli*. The least sensitive to the effect of 10*H*-[1,2,4]triazino[5,4-*b*]quinazolin-10-ones was *P. aeruginosa*. Only derivative 120 exhibited the total inhibition of the growth of *P. aeruginosa* (MIC = 100 mg/L). Other 10*H*-[1,2,4]triazino[5,4-*b*]quinazolin-10-ones were inactive.

The data presented in Table 7 show the antibacterial activity of quinazoline derivatives comprising amino acid moiety at position 3. All the test compounds were found to be non-effective. The MIC values were higher than the highest concentration tested.

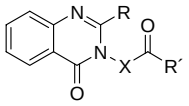
The effect of [1,2,4]triazoloquinazolines is summarized in Table 8. The broadest antibacterial spectrum was encountered with derivative 142 which was effective with all tested bacteria (MIC = 10-100 mg/L). Derivative 140 was effective with the three tested bacteria. It was more active against *S. aureus* and *E. coli* than ampicillin. Derivative 142 was more active against *P. aeruginosa* than ampicillin. *S. aureus* was found to be the most sensitive bacterium towards the quinazolines presented in Table 8. Derivatives 140, 142, 144 and 145 manifested antibacterial activity on *S. aureus*.

The MIC values of tetrazolo[1,5-*c*]quinazolines are given in Table 9. Sensitivity of Gram positive bacteria to the derivatives was higher than that of Gram negative bacteria. Derivative 155 was effective against *S. aureus* and *P. aeruginosa* (MIC= 1000 mg/L). None of tetrazolo[1,5-c]quinazolines influenced *E. coli*.

The results presented in Table 10 show the antibacterial activity of the two triazino[4,3-*c*]quinazolines. None of them influenced the bacterial growth (MIC > 100 mg/L).

The data presented in Tables 1–10 imply

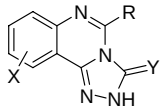
Table 7. Antibacterial activity of some 4-oxoquinazolines containing amino acid or amino ester moieties. [a]



No.	R	X	R'	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
122	Methyl	-CH_2-	OH	>100	>100	>100	>100
123	Methyl	1,4-(C_6H_4)-	OH	>100	>100	>100	>100
124	Phenyl	-CH_2CH_2-	OH	>100	>100	>100	>100
125	Pheny	1,4-(C_6H_4)-	OH	>100	>100	>100	>100
126	Methyl	-CH_2-	NH-CH(COOH)-CH_2-CH_2-COOH	>100	>100	>100	>100
127	Methyl	-CH_2CH_2-	NH-CH(COOH)-CH_2-CH_2-COOH	>100	>100	>100	>100
128	Methyl	1,4-(C_6H_4)-	NH-CH(COOH)-CH_2-CH_2-COOH	>100	>100	>100	>100
129	Phenyl	1,4-(C_6H_4)-	NH-CH(COOH)-CH_2-CH_2-COOH	>100	>100	>100	>100
130	Methyl	-CH_2-	CH_2-COOH	>100	>100	>100	>100
131	Methyl	-CH_2-	CH_2CH_2-COOH	>100	>100	>100	>100
132	Methyl	-CH_2CH_2-	CH_2-COOH	>100	>100	>100	>100
133	Methyl	-CH_2	NH-CH(COOEt)-CH_2-CH_2-COOEt	>100	>100	>100	>100
134	Methyl	1,4-(C_6H_4)-	NH-CH(COOMe)-CH_2-CH_2-COOMe	>100	>100	>100	>100
135	Phenyl	-CH_2CH_2-	NH-CH(COOEt)-CH_2-CH_2-COOEt	>100	>100	>100	>100
136	Phenyl	1,4-(C_6H_4)-	NH-CH(COOMe)-CH_2-CH_2-COOMe	>100	>100	>100	>100
137	H	–	NH-CH_2-COOH	>100	>100	>100	>100
138	H	–	NH-CH_2-CH_2-COOH	>100	>100	>100	>100
Amp				1**	>100	0.04**	10**

[a] Values of MIC are in mg/L; Amp-ampicillin; Me = methyl, Et = ethyl;** bactericidal effect.

Table 8. Antibacterial activity of some 1,2,4-triazolo[4,3-c]quinazoline-3-thiones, resp. 3-ones. [a]



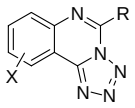
No.	X	R	Y	E. coli[b]	*P. aeruginosa*[b]	*S. aureus*[b]	*B. subtilis*[b]
139	9-Cl	Phenyl	S	>200	>200	>200	>200
140	9-Cl	Morpholin-4-yl	S	1*	>200	0.1–1	0.1–1
141	9-Cl	Piperidin-1-yl	S	>200	>200	>200	>200
142	9-Cl	4-Phenylpiperazin-1-yl	S	10–100	10–100	10–100	10–100
143	7-Me	Phenyl	S	>200	>200	>200	>200
144	H	H	O	>100	>100	100	>100
145	H	Phenyl	O	>100	>100	50	>100
146	H	Methyl	O	>100	>100	>100	>100
Amp				1**	>100	0.04**	10**

[a] Values of MIC are in mg/L; Amp-ampicillin; ** bactericidal effect, * bacteriostatic effect.
[b] Reported in Jantová et al. (1994b).

that, in general, the sensitivity of the Gram positive bacteria to the quinazolines tested was higher than that of Gram negative bacteria.

The [1,2,4]triazoloquinazolines (Tab. 8) and 10*H*-[1,2,4]triazino[5,4-*b*]quinazolin-10-ones (Tab. 6) were found to be the most effective among the quinazolines tested. The minimal inhibition concentration of five 10*H*-[1,2,4]triazino[5,4-*b*]quinazolin-10-ones for *B. subtilis* was lower than the corresponding value of ampicillin and was in the range 1–5 mg/L. The MIC values of the most effective derivatives were in the range of 10–100 mg/L for *S. aureus* and in the range 5–10 mg/L for *E. coli*. The most effective derivative 1-[(3-methylphenyl)amino]-10*H*-[1,2,4]triazino[5,4-*b*]quinazolin-10-one (120) had

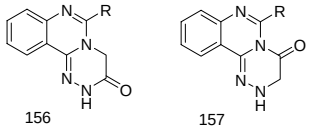
Table 9. Antibacterial activity of some tetrazolo[1,5-c]quinazolines. [a]



No	X	R	E. coli[b]	P. aeruginosa[b]	S. aureus[b]	E. faecalis[b]
147	H	Diphenylamine	>1000	>1000	>1000	>1000
148	9-Cl	Morpholin-4-yl	>1000	>1000	>1000	>1000
149	9-Br	Morpholin-4-yl	>1000	>1000	>1000	>1000
150	9-Cl	Piperidin-1-yl	>1000	>1000	>1000	>1000
151	H	Morpholin-4-yl	>1000	>1000	>1000	>1000
152	H	Phenyl	>1000	>1000	250*	250*
153	9-Cl	Phenyl	>1000	>1000	1000*	1000*
154	9-Br	Phenyl	>1000	>1000	>1000	>1000
155	7-CH_3	Phenyl	>1000	1000	1000	>1000
Amp			1**	>500	0.04**	1**

[a] Values of MIC are in mg/L; Amp-ampicillin; ** bactericidal effect, * bacteriostatic effect.
[b] Reported in Jantová et al. (2004).

Table 10. Antibacterial activity of some triazino[4,3-c]quinazoline-4-ones. [a]



156 157

No.	R	E. coli[b]	P. aeruginosa[b]	S. aureus[b]	B. subtilis[b]
156	Methyl	>100	>100	>100	>100
157	Phenyl	>100	>100	>100	>100
Amp		1**	>100	0.04**	10**

[a] Values of MIC are in mg/L; Amp-ampicillin; ** bactericidal effect.
[b] Reported in Jantová et al. (1999).

displayed MIC value of 5 mg/L for *E. coli*, 100 mg/L for *P. aeruginosa*, 10 mg/L for *S. aureus*, and 1 mg/L for *B. subtilis*.

Among the [1,2,4]triazoloquinazolines the most effective derivatives showed the MIC values in the range of 0.1-100 mg/L for *S. aureus* and *B. subtilis* and 1–100 mg/L for *E. coli*. The most effective derivative 9-chloro-morpholin-4-yl [1,2,4]triazolo[4,3-*c*]quinazolin-3(4*H*)-thione (140) demonstrated the MIC value lower than ampicillin for *B. subtilis* and the same MIC value for *E. coli*.

The condensed quinazolines (120, 140) were highly effective, too. The MIC values of the most effective quinazolines were in the range of 0.1-10.0 mg/L and they were higher than those of the standard.

Discussion

Infectious diseases caused by bacteria affect millions of people worldwide. Concerted and systematic programs to discover and develop new antibiotics have been driven to a considerable extent by the development of resistance by these organisms to the drugs commonly used against them.

In the present study the antibacterial activity of 157 substituted quinazolines against bacterial strains *Escherichia coli* CCM 3988, *Pseudomonas aeruginosa* CCM 3955, *Bacillus subtilis* ATCC 6663 and *Staphylococcus aureus* CCM 3953 by microdilution assay was investigated. The minimum inhibitory concentration (MIC) of the quinazolines tested has been determined by a microdilution method in 96-well microtitration plates.

The structure-activity relationship has also been studied. Ten series of substituted quinazolines were tested, namely the substituted quinazoline-4(3*H*)-ones and their thioanalogues (Tab. 1); 2,3-disubstituted quinazoline-4(3*H*)-ones and thiones (Tab. 2); 4-quinazolylhydrazines their arylhydrazones and 4-quinazolylthiosemicarbazides (Tab. 3); 2,6-disubstituted 4-anilino-quinazolines (Tab. 4); 1,2,4-triazolo[4,3-*c*]quinazolines (Tab. 5); 10*H*-[1, 2,4]triazino[5,4-b]quinazolin-10-ones (Tab. 6); quinazolines with aminoacid groups at position 3 (Tab. 7); [1,2,4]triazoloquinazolines (Tab. 8); tetrazolo[1,5-*c*] quinazolines (Tab. 9); and triazino[4,3-*c*]quinazolines (Tab. 10).

In the first stage of the preparation of quinazoline derivatives, simple quinazolines were synthesized, e.g. those substituted in positions 2, 3 and 4 of the pyrimidine ring, as well as in various positions of the benzene ring by substituents known as pharmacophores (Tabs 1, 2, 3, 4 and 7). In the next stage, condensed quinazolines were prepared and tested (Tabs 5, 6, 8, 9 and 10). Further five- and six-membered rings containing several nitrogen atoms were fused with the "b" or "c" side of the pyrimidine ring of quinazoline skeleton in expectation of substantial change in bioactivity. It turned out, that the most effective quinazolines were condensed [1,2,4]triazoloquinazolines (Tab. 8) and 10*H*-[1,2,4]triazino[5,4-*b*]quinazolin-10-ones (Tab. 6). Our examination of structure-activity relationship further showed that the most effective derivatives were those carrying an unsubstituted benzene ring or one substituted with small substituents (Cl and CH_3) while having pyrimidine ring substituted with larger substituents, such as morpholine, phenyl or secondary amines. The most effective quinazolines had small molecular size.

The highest antimicrobial activity was evoked by substitution with chloro or methyl groups at the 4-position of the aryl ring of substituted 4(3*H*)-quinazolone derivatives synthesized by Misra et al.(1982), too.

Similarly Kuyper et al. (1996) found, that antimicrobial activity of 7,8-dialkyl-1,3-diaminopyrrolo[3,2-*f*]quinazolines might be inversely related to the molecular size of the inhibitor. Their results suggested that relatively small chemical group would provide optimal interactions with a hydrophobic region of dihydrofolate reductase.

Bekhit et al. (2001) synthesized and evaluated antibacterial and antifungal activity of new chalcone and sydnone derivatives of 4(3*H*)-quinazolinone. The most potent compound was derivative with small chemical group – the nitroso derivative.

Furthermore our results showed that the Gram positive bacteria were more sensitive that Gram negative bacteria towards the tested quinazolines. The higher sensitivity of Gram positive bacteria relative to Gram negative bacteria was proved by Elslager et al. (1978 a,b), Khalil & Habib (1987), Baiocchi et al.(1993) and Shiba et al. (1997). On the other hand, Aboushady at al. (1979), Elslager et al. (1984), Farghaly et al. (1990) and Nasr et al. (2003) reported that the tested quinazolines were active on Gram positive and Gram negative bacteria. It probably relates with data, that some quinazolines are species selective inhibitors of dihydrofolate reductase (Chan et al., 1995).

From the preliminary results of this study we could conclude that 1-[(3-methylphenyl)amino]-10*H*-[1,2,4] triazino[5,4-*b*]quinazolin-10-one (120) and 9-chloro-morpholin-4-yl [1,2,4]triazolo[4,3-*c*] quinazolin- 3(4*H*)-thione (140) are the most active antibacterial agents.

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