(c = 0.5 mol dm^{-3}, 0.2 cm^3) was thoroughly mixed with ethyl acetate (2.0 cm^3, 5 min), suspension was centrifuged (10 000 min^{-1}, 3 min), and 3 mm^3 of the supernatant were injected onto the chromatographic column. A linear relationship between peak area and concentration of the determined compounds in the range of 20—300 μg cm^{-3} was observed with the regression coefficient r better than 0.985 for both compounds. Results were calculated for n = 5, α = 0.05.

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Translated by B. Proksa

Azolylquinazolines, Synthesis and Biological Activity

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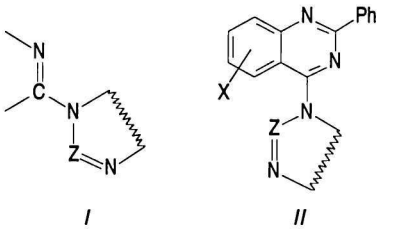
Received 23 December 1993

Preparation of some 2-phenyl-4-(azol-1-yl)quinazolines by reaction of corresponding chloroquinazolines with sodium salt of azoles is described. The IR, UV, and ^{1}H NMR spectra and the preliminary screening of biological activity of final products are presented.

The systems in which the 1,2,4-triazole skeleton is connected to the imidoyl grouping (*I*) showed a number of interesting biological effects. The phytoeffectorial one is the most significant of them [1, 2].

In order to extend the series of these compounds, the present communication describes syntheses of some 4-(azol-1-yl)quinazolines (*II*) in which imidoyl grouping is latent being the part of pyrimidine skeleton. At the same time, for the reason of biological activity chlorine was introduced at the benzene ring (Formulae 1).

The syntheses of final compounds started from the chosen 2-phenyl-4-chloro-, 2-phenyl-4,6-dichloro-, and 2-phenyl-4,6,8-trichloroquinazolines, respectively, obtained by the classic methods using anthranilic acid as starting material [3—7].



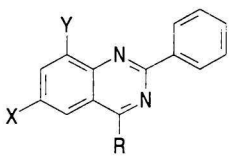
I *II*

Z = N, CH

X = H, 6-Cl, 6,8-diCl

Formulae 1

The direct nucleophilic substitution of chlorine in position 4 by sodium salt of corresponding azole afforded 2-phenyl-4-(azol-1-yl)quinazolines *IIIa—IIIℓ*.



IIIa—IIIℓ

	X	Y	R
a	H	H	imidazole
b	H	H	benzimidazole
c	H	H	triazole
d	H	H	benzotriazole
e	Cl	H	imidazole
f	Cl	H	benzimidazole
g	Cl	H	triazole
h	Cl	H	benzotriazole
i	Cl	Cl	imidazole
j	Cl	Cl	benzimidazole
k	Cl	Cl	triazole
l	Cl	Cl	benzotriazole

Characterization of the prepared compounds is given in Table 1.

The IR spectra (Table 2) revealed characteristic bands at $\tilde{\nu}$ = 1590—1620 cm^{-1} associated with ν(C=N) vibration. Determination of typical vibrations of individual azoles was realized by comparison with the IR data reported [8].

The UV spectra (Table 2) displayed the absorption maxima in the region λ = 200—350 nm. Comparison with the data reported [8] showed that the absorption maxima λ = 209—214 nm belong to the imidazole (*IIIa, IIIe, IIIi*) and absorption bands at λ = 263—267 nm to the benzimidazole (*IIIb, IIIf, IIIj*). The compounds *IIIc, IIIg, IIIk* showed bands at λ = 210—215 nm and the compounds *IIId, IIIh, IIIℓ* showed bands at λ = 253 nm, 256 nm, 266 nm, which were assigned to the triazole and benzotriazole rings. All of these absorption maxima had a high value of molar extinction coefficient (log {ε} ≈ 3.5). Also, most of these compounds exhibited the presence of bands at λ = 330—350 nm, which demonstrated the connection of azoles to the conjugation with quinazoline skeleton.

The ^{1}H NMR spectra of prepared compounds *IIIa—IIIℓ* (Table 3) showed typical signals of azoles in the region δ = 8.4—9 Multiplets of phenyl protons were found at δ = 7—8 and multiplets of quinazoline skeleton protons at δ = 7.8—8.5.

The preliminary screening of biological activity of prepared derivatives has shown certain antimicrobial and cytotoxic activity of some of them. The widest antimicrobial activity has been manifested by the derivative *IIIa,* which was effective with *Escherichia coli*, *Sarcinia flava,* and *Bacillus subtilis*, and with all tested filamentous micromycetes. Certain antibacterial activity as well as effects on phytopathogenic fungus *Fusarium nivale* has been manifested by the

Table 1. Characterization of the Compounds *IIIa—IIIℓ*

Compound	Formula M_r	w_i(calc.)/% w_i(found)/%			Yield/%	M.p./°C
		C	H	N		
IIIa	$C_{17}H_{12}N_4$ 272.3	74.98 74.78	4.44 4.41	20.57 20.36	77	152—154
IIIb	$C_{21}H_{14}N_4$ 322.4	78.24 78.09	4.38 4.29	17.38 17.22	70	182—183
IIIc	$C_{16}H_{11}N_5$ 273.3	70.32 70.21	4.06 4.01	25.63 25.52	45	154—155
IIId	$C_{20}H_{13}N_5$ 323.4	74.29 74.11	4.05 4.00	21.66 21.58	60	199—200
IIIe	$C_{17}H_{11}N_4Cl$ 306.8	66.56 66.47	3.61 3.54	18.26 18.13	55	104—105
IIIf	$C_{21}H_{13}N_4Cl$ 356.8	70.69 70.55	3.67 3.56	15.70 15.61	32	92—95
IIIg	$C_{16}H_{10}N_5Cl$ 307.7	62.45 62.33	3.28 3.19	22.76 22.66	32	110—111
IIIh	$C_{20}H_{12}N_5Cl$ 357.8	67.14 66.99	3.38 3.36	19.57 19.44	54	105—106
IIIi	$C_{17}H_{10}N_4Cl_2$ 341.2	59.84 59.80	2.95 2.91	16.42 16.37	46	146—149
IIIj	$C_{21}H_{12}N_4Cl_2$ 391.3	64.47 64.38	3.09 3.02	14.32 14.25	40	231—235
IIIk	$C_{16}H_9N_5Cl_2$ 342.2	56.16 56.09	2.65 2.61	20.47 20.33	50	111—114
IIIℓ	$C_{20}H_{11}N_5Cl_2$ 392.2	61.24 61.18	2.83 2.79	17.85 17.77	40	116—118

Table 2. Spectral Data of the Compounds *IIIa*—*IIIℓ*

Compound	$\tilde{\nu}$/cm^{-1}			λ_{max}/nm					
	ν(C—H)	ν(C=N)	ν(C=C)	log {ε}					
IIIa	3096	1614	1570	209	225	266	333		
	3061		1564	3.58	3.17	3.60	2.65		
IIIb	3096	1618	1568	205	228	266			
	3061	1591		3.76	3.19	3.60			
IIIc	3059	1616	1583	210	224	270	337		
			1574	3.50	3.09	3.60	2.68		
IIId	3059	1618	1579	202	226	266	341		
		1597	1568	3.83	3.31	3.72	3.06		
IIIe	3022	1618	1574	209	229	249	267	289	333
			1562	3.41	3.05	3.35	2.93	3.18	2.34
			1558						
IIIf	3063	1618	1572	208	229	249	267	289	333
	3022			3.67	3.28	3.60	3.18	3.43	2.56
IIIg	3053	1612	1570	210	229	252	267	289	
	3020		1560	3.56	3.20	3.52	3.17	3.34	
IIIh	3063	1614	1587	210	230	253	267	289	
	3032		1568	3.67	3.30	3.63	3.29	3.44	
IIIi	3084	1608	1587	214	230	262	274	297	
	3063	1601	1562	3.46	3.19	3.58	3.08	3.32	
IIIj	3086	1593	1556	200	233	263	352		
	3059			3.87	3.37	3.65	2.94		
IIIk	3092	1589	1564	215	230	256	281	296	333
				3.46	3.20	3.56	3.24	3.28	2.75
IIIℓ	3088	1593	1560	204	230	256	278	296	333
	3063			3.51	3.24	3.56	3.26	3.29	3.81

derivative *IIId*. The quinazolines *IIIf*, *IIIi*, and *IIIj* influenced only G^+ bacteria. None of the prepared derivatives influenced the G^- *Pseudomonas fluorescens* and the tested yeasts. The derivative *IIIg* (ID_{50} = 19 μg cm^{-3}) has manifested the highest cytotoxic activity on the tumour HeLa cells. The substances *IIIa*, *IIIb*, and *IIIf* (ID_{50} = 21.34 μg cm^{-3} and 45 μg cm^{-3}) have been less influential. ID_{50} is such a concentration of a derivative which in comparison with the control inhibits the content of total proteins in a cell to 50 %.

From the viewpoint of evaluating the biological activity and substitution in the aromatic ring of the quinazoline skeleton, the derivatives can be classified into nonsubstituted, substituted by chlorine in position 6, and substituted by chlorine in positions 6 and 8. It can be seen in Table 4 that all three types of the compounds influence HeLa cells. The filamentous fungi were affected only by the nonsubstituted derivatives. As for azoles, the widest activity on the tested material has been manifested

Table 3. ^{1}H NMR Data of the Compounds *IIIa*—*IIIℓ*

Compound	δ		
	Azole	Quinazoline	Phenyl
IIIa	8.89 (s, 1H) 8.52—8.57 (m, 2H)	7.85—8.16 (m, 4H)	7.36—7.61 (m, 5H)
IIIb	8.45—8.62 (m, 5H)	8.12—8.40 (m, 4H)	7.34—8.08 (m, 5H)
IIIc	9.89 (s, 1H) 9.06—9.16 (d, 1H)	8.36—8.70 (m, 4H)	7.55—8.26 (m, 5H)
IIId	8.82—8.93 (d, 1H) 8.45—8.57 (m, 3H)	7.94—8.17 (m, 4H)	7.56—7.90 (m, 5H)
IIIe	8.50—8.75 (m, 3H)	8.05—8.20 (m, 3H)	7.65—7.70 (m, 5H)
IIIf	8.44—8.57 (m, 5H)	7.92—8.05 (m, 3H)	7.51—7.56 (m, 5H)
IIIg	8.44—8.56 (m, 2H)	7.95—8.11 (m, 3H)	7.51—7.59 (m, 5H)
IIIh	8.43—8.56 (m, 4H)	7.94—8.10 (m, 3H)	7.51—7.64 (m, 5H)
IIIi	8.44—8.52 (m, 3H)	8.13 (bs, 1H) 7.94 (s, 1H)	7.57—7.60 (m, 5H)
IIIj	9.02 (s, 1H) 8.65 (m, 4H)	8.25 (s, 1H) 7.95 (s, 1H)	7.60—7.75 (m, 5H)
IIIk	8.50—8.55 (m, 2H)	8.09—8.21 (m, 2H)	7.49—7.86 (m, 5H)
IIIℓ	8.35—8.42 (m, 4H)	7.82—7.97 (m, 2H)	7.48—7.65 (m, 5H)

Table 4. Biological Activity of the Compounds *IIIa—IIIℓ*

Compound	Φ/mm[a]													ID_{50} /μg cm^{-3}
	Bacteria					Yeasts				Filamentous fungi				HeLa cells
	E. coli	*P. fluorescens*	*B. subtilis*	*S. flava*	*S. aureus*	*S. cerevisiae*	*C. albicans*	*C. tropicalis*	*C. parapsilosis*	*R. nigricans*	*A.alternata*	*A. niger*	*F. nivale*	
IIIa	16	–	22	32	–	–	–	–	–	30	30	11	20	34
IIIb	–	–	–	–	–	–	–	–	–	–	–	–	–	45
IIIc	–	–	–	–	–	–	–	–	–	–	–	–	–	> 50
IIId	–	–	–	16	–	–	–	–	–	–	–	–	14	> 50
IIIe	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IIIf	–	–	–	16	–	–	–	–	–	–	–	–	–	21
IIIg	–	–	–	–	–	–	–	–	–	–	–	–	–	19
IIIh	–	–	–	–	–	–	–	–	–	–	–	–	–	> 50
IIIi	–	–	–	20	18	–	–	–	–	–	–	–	–	> 50
IIIj	–	–	–	–	14	–	–	–	–	–	–	–	–	> 50
IIIk	–	–	–	–	–	–	–	–	–	–	–	–	–	> 50
IIIℓ	–	–	–	–	–	–	–	–	–	–	–	–	–	> 50

a) Φ — diameter of the sterile inhibition zone. – Sterile inhibition zone has not occurred (inactive compound).

by the imidazole derivatives; out of these the basic derivative *IIIa* has been the most effective from all the studied compounds. On the other hand, the condensed analogues, *i.e.* 4-(1-benzimidazolyl)quinazolines have not been biologically active.

EXPERIMENTAL

^{1}H NMR spectra of hexadeuterodimethyl sulfoxide solution were taken with the spectrometer Tesla BS 487 C (80 MHz) using tetramethylsilane as internal standard. The IR spectra of compounds in KBr pellets were measured with a Philips PU 9800 FTIR. Ultraviolet spectra of methanolic solutions ($c = 10^{-4}$ mol dm^{-3} in a 0.2 cm cell) were taken with a Specord M 40 (Zeiss, Jena) instrument. The starting compounds were prepared according to the literature: 5-chloro- and 3,5-dichloroanthranilic acid [3], 5-chloro- and 3,5-dichloro-*N*-benzoylanthranilic acid [4], substituted *N*-benzoylanthranilic acid amides [5], 2-phenylquinazolone [6], and 2-phenyl-4-chloroquinazoline [7].

The antimicrobial activity of prepared quinazoline derivatives was evaluated using the G$^-$ bacteria *Escherichia coli* and *Pseudomonas fluorescens*; the G$^+$ bacteria *Sarcina flava*, *Staphylococcus aureus,* and *Bacillus subtilis*; the yeasts *Saccharomyces cerevisiae, Candida albicans, Candida tropicalis,* and *Candida parapsilosis*; the filamentous fungi *Rhizopus nigricans, Alternaria alternata, Aspergillus niger,* and *Fusarium nivale*. In preliminary tests antibacterial and antifungal activity of the compounds was assayed by a paper disk diffusion technique [9]. The antimicrobial activity of the tested quinazoline derivatives was evaluated from the diameter of the sterile inhibition zone which occurred around the centre of diffusion of the effective derivatives.

The cytotoxic activity of the prepared derivatives was studied on the transformed tumour cell line HeLa. A three-day culture of HeLa cells was trypsinized and was used to prepare a suspension with concentration 3.5×10^{-4} cells cm^{-3}. The experiments were carried out in Leighton flasks into which 2 cm^3 of the above-mentioned suspension were pipetted. After 24 h of static culturing at 37 °C, the substances, previously dissolved in dimethyl sulfoxide, were gradually added in five different concentrations (in the range of 0.01—100 μg cm^{-3}), 20 mm^3 of each per culturing flask. First, the effect of the substances on cell morphology was microscopically evaluated after 48 h of incubation at 37 °C. Then, the intensity of growth of the cells was evaluated by the Lowry method stating the content of total cell protein [10]. The cytotoxic activity of the derivatives was stated from inhibitory doses ID_{50} which were read out from the toxicity curves.

[a]2-Phenyl-6-chloroquinazolone and [b]2-Phenyl-6,8-dichloroquinazolone

Corresponding *N*-benzoylanthranilic acid amide (0.05 mol) was heated at 240—250 °C for 45 min. Then the melted solid was cooled down to room temperature and crystallized from ethyl acetate.

a: m.p. = 295—300 °C, yield 62 %. For $C_{14}H_9ClN_2O$ (M_r = 256.69) w_i(calc.): 13.81 % Cl, 10.91 % N; w_i(found): 13.78 % Cl, 10.79 % N.

b: m.p. = > 300 °C, yield 69 %. For $C_{14}H_8Cl_2N_2O$ (M_r = 291.14) w_i(calc.): 24.35 % Cl, 9.62 % N; w_i(found): 24.23 % Cl, 9.58 % N.

[c]2-Phenyl-4,6-dichloroquinazoline and [d]2-Phenyl-4,6,8-trichloroquinazoline

The mixture of the corresponding quinazolone (0.02 mol), $POCl_3$ (6 cm^3), *N,N*-dimethylaniline (9 cm^3), and dry benzene (100 cm^3) was refluxed for 2 h. After cooling 100 cm^3 of benzene was added, the diluted mixture was washed with water, then with 10 % solution of Na_2CO_3 and water. The benzene layer was dried, concentrated and left to crystallize. The raw product was recrystallized from acetonitrile.

c: m.p. = 140 °C, yield 81 %. For $C_{14}H_8Cl_2N_2$ (M_r = 275.15) w_i(calc.): 25.77 % Cl, 10.18 % N; w_i(found): 25.72 % Cl, 10.09 % N.

d: m.p. = 160—161 °C, yield 80 %. For $C_{14}H_7Cl_3N_2$ (M_r = 309.58) w_i(calc.): 34.36 % Cl, 9.05 % N; w_i(found): 34.28 % Cl, 8.95 % N.

2-Phenyl-4-(azol-1-yl)quinazolines *IIIa—IIIℓ*

To chloroquinazoline (0.005 mol) dissolved in 50 cm^3 of absolute acetonitrile sodium salt (0.005 mol) of corresponding azole was added. The reaction mixture was stirred and refluxed for 12 h, then filtered. After filtration, the product was obtained by cooling.

Characteristic data of prepared compounds are presented in Tables 1—4.

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Translated by K. Špirková