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SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 1,2,3,4-TETRAHYDRO- AND PYRIDO[1,2-*a*]BENZIMIDAZOLES

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We report here the synthesis of a series of different 1,2,3,4-tetrahydro- and pyrido[1,2-*a*]benzimidazoles and assessment of their *in vitro* antibacterial activity against Gram-positive (*Bacillus cereus*) and Gram-negative (*Escherichia coli* AB1157, *Pseudomonas aeruginosa* PAO1) bacteria. Pyrido[1,2-*a*]benzimidazoles were the most effective against *Bacillus cereus*, while 1,2,3,4-tetrahydro derivatives produced the greatest level of growth inhibition of Gram-negative (*Escherichia coli* AB1157) bacteria. The antibacterial activity of 2,4-dimethyl-7,8-dinitropyrido[1,2-*a*]benzimidazole was comparable to or exceeded the efficacy of commercial tetracycline, kanamycin, levomycetin, and erythromycin formulations. A genotoxicology test (the *Allium* test) was used to study the mitosis-modifying and mutagenic actions of a series of condensed azaheterocyclic compounds with marked antibacterial effects. On the basis of the antibacterial activity of some of the condensed benzimidazole derivatives containing a nodal nitrogen atom detected here and the low toxicity of similar azaheterocycles led to the conclusion that there is potential for further searches for novel antibiotics among compounds of this class.

Keywords: pyrido[1,2-*a*] benzimidazoles, 1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazoles, antibacterial activity, mitotic and phasic indexes, anaphase-telophase analysis.

Bacterial infectious diseases remain among the leading causes of death throughout the world [1]. The antibiotics used for treating them lose efficacy over time because of the development of resistance in bacteria [2, 3]. The result is that treatment of resistant microorganisms becomes more problematic and requires the use of new drugs. Studies of the synthesis of novel biologically active compounds and studies of their antibacterial activity are constantly ongoing.

Many researchers seeking novel antibiotics have in recent years been attracted to various condensed derivatives of benzimidazole with nodal nitrogen atoms, such as pyrido[1,2-*a*]benzimidazoles. This is associated with the fact that such substances have high antimicrobial activity against a variety of bacterial species [4 – 10].

Thus, series of compounds have been found to be active against Gram-negative *Escherichia coli* [4, 7 – 10], *Salmonella typhi* [7 – 9], and *Vibrio cholerae* [7, 8] and Gram-positive *Bacillus subtilis* [6 – 8], *Clostridium tetani* [6, 8], *Streptococcus pneumoniae* [6], *Staphylococcus aureus* [4, 9, 10], and *Mycobacterium tuberculosis* [5]. At the same time, the literature lacks data on the antimicrobial activity of tetrahydro derivatives of pyrido[1,2-*a*]benzimidazole and the effects of substituents in the benzene ring of this condensed heterocycle on their activity have received virtually no study.

Thus, we report here the synthesis of a wide range of condensed benzimidazole derivatives containing pyridine (**I-XVII**) or piperidine (**XVIII-XXI**) rings annealed to the imidazole moiety; we also report the first studies of their antibacterial activity. The mitosis-modifying and mutagenic actions of the substances with the greatest antibacterial effects were also studied.

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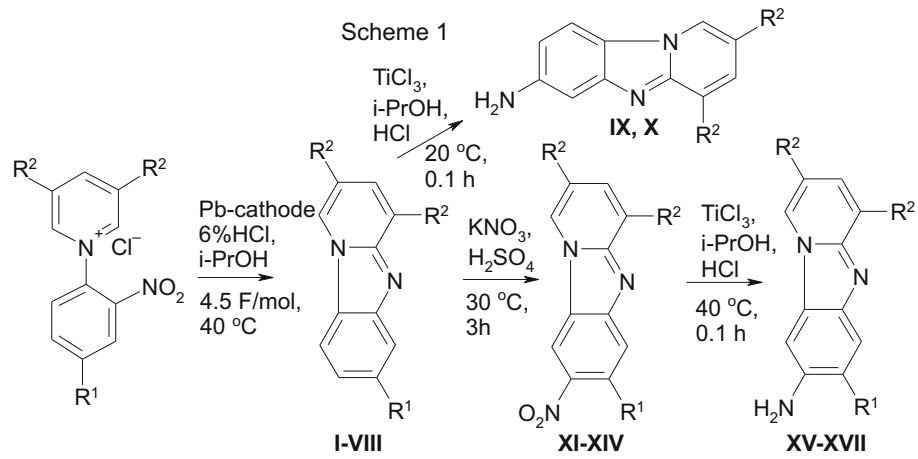
Scheme 1



I: $R^1 = R^2 = H$; **II**: $R^1 = CF_3$, $R^2 = H$; **III**: $R^1 = COOEt$, $R^2 = H$; **IV**: $R^1 = CN$, $R^2 = H$; **V**: $R^1 = NO_2$, $R^2 = H$; **VI**: $R^1 = Cl$, $R^2 = H$; **VII**: $R^1 = CN$, $R^2 = CH_3$; **VIII**: $R^1 = NO_2$, $R^2 = CH_3$; **IX**: $R^2 = H$; **X**: $R^2 = CH_3$; **XI**: $R^1 = CF_3$, $R^2 = H$; **XII**: $R^1 = COOEt$, $R^2 = H$; **XIII**: $R^1 = NO_2$, $R^2 = H$; **XIV**: $R^1 = NO_2$, $R^2 = CH_3$; **XV**: $R^1 = CF_3$, $R^2 = H$; **XVI**: $R^1 = COOEt$, $R^2 = H$; **XVII**: $R^1 = NH_2$, $R^2 = H$.

EXPERIMENTAL CHEMICAL SECTION

Melting temperatures were measured on a PolyTherm A apparatus with a heating rate of 3°C/min and were not corrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 spectrometer, SF = 400 MHz, solvent DMSO-d_6, temperature 25°C. The standard for determining chemical shifts consisted of residual proton signals from the solvent in proton spectra (2.5 ppm) or DMSO-d_6 signals in carbon spectra (39.5 ppm. High-resolution mass spectra were recorded on a Bruker micrOTOF II apparatus (Bruker Deltronics) with electrospray ionization (ESI) and a mass scanning range (*m*/*z*) of 3000 Da, with samples injected by syringe. The solvent was MeCN and the solvent flow rate was 3 μl/min. The interface temperature was 180°C and the carrier gas was nitrogen (4.0 liters/min).

Pyrido[1,2-*a*]benzimidazoles (**I-VIII**) and 1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazoles (**XVIII-XXI**) were synthesized as described in [11].

Nitro derivatives of pyrido[1,2-*a*]benzimidazole (**XI-XIV**) were synthesized as described in [12].

Amino derivatives of pyrido[1,2-*a*]benzimidazole (**IX**, **X**, **XV-XVII**) were synthesized as described in [13].

EXPERIMENTAL BIOLOGICAL SECTION

The antibacterial activity of pyrido[1,2-*a*]benzimidazoles (**I-XVII**) and 1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazoles (**XVIII-XXI**) was studied using a standard method [14] on microorganisms widely used for developing antibiotics. Tests used two Gram-negative strains (*Escherichia coli* AB1157 and *Pseudomonas aeruginosa* PAO1, from the collection of the Institute of Molecular Genetics, provided by the Kurchatov Institute, Moscow) and one Gram-positive strain (*Bacillus cereus*, from the collection of the Department of Botany and Microbiology, Yaroslavl State University).

The antibacterial activity of pyrido[1,2-*a*]benzimidazole derivatives was assessed using commercial antibiotics used against Gram-negative and Gram-positive bacteria: gentamicin, tetracycline, kanamycin, levomycetin, and erythromycin.

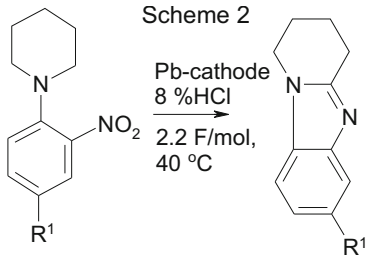
Testing by the serial dilutions method was run in sterile 96-well immunology plates in volumes of 0.2 ml with final microorganism concentrations of 10^6 cfu/ml. Minimal inhibitory concentration (MIC) were determined for test compounds, at which they completely suppressed bacterial growth at 24 h of incubation at 37°C. Bacterial growth was assessed by measuring absorption at 600 nm with a Bio-Rad iMark photometric plate reader.

Mitosis-modifying and mutagenic properties of the compounds with the greatest antibacterial activity were studied using a genotoxicity test (the *Allium* test) on meristem cells from *Allium cepa* sprout root cells as described in [15]. Commercial antibiotics – tetracycline and gentamycin – were used as reference compounds.

Mutagenic activity was evaluated in terms of the ability of substances to induce chromosomal aberrations at the anaphase-telophase stage. Factors assessed were: the total number of cells (≈600), the total number of dividing cells at the anaphase-telophase stage, and the number of cells with chromosomal aberrations.

Mitotoxic activity and mitosis-modifying actions were assessed in the same time-based preparations as anaphase-telophase analysis. The mitosis-modifying actions of compounds were evaluated in terms of the level of mitotic activity in tissues – the mitotic index (MI, %) – and phase duration indexes. The total number of cells (≈600), the number of nondividing cells, and the numbers of cells at different phases of mitosis were determined.

Scheme 2



XVIII-XXI

XVIII: R^1 = H; **XIX**: R^1 = CF_3; **XX**: R^1 = CN; **XXI**: R^1 = Cl.

Significant differences would experiments and controls were identified by two-factor analysis of variance and Student's test. Differences were taken as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Pyrido[1,2-*a*]benzimidazoles (**I-VIII**) were synthesized by reductive intramolecular heterocyclization of quaternary N-(2-nitro(phenyl)aryl) pyridinium salts (Scheme 1). The electron source was an electric current, use of which significantly reduced the cost of synthesis and excluded formation of toxic wastes. The extraction procedure was also simplified, as the reaction material at the end of the reaction contained only the reaction product, ethanol, and the background electrolyte, which was 6% HCl. Syntheses were run at a temperature of 40°C by passage of 4.5 F/mol of electricity through an electrolytic cell. The yields of products **I-VIII** were 85 – 96%.

Nitration and reduction reactions yielded pyrido-[1,2-*a*]benzimidazoles (**XI-XVII**), which contained both an electron-donor or electron-acceptor substituent or substituents of different electronic nature. The nitrating agent was a mixture of KNO_3 and H_2SO_4. Reactions were run at 30°C for 3 h. Nitro products **XI-XIV** were extracted with yields of 89 – 94%. Reduction of nitro compounds **V**, **VIII**, and **XI-XIV** was with titanium (III) chloride, which produced contaminant-free amines **IX**, **X**, **XV-XVII** with yields of 91 – 97%.

1,2,3,4-Tetrahydropyrido[1,2-*a*]benzimidazoles (**XVIII-XXI**) were also synthesized by reductive intramolecular cyclization (Scheme 2). By comparison with pyrido[1,2-*a*]-benzimidazoles (**I-VIII**), these reactions required passage of half the quantity of electricity. Electrolyte was 8% HCl containing N-(2-nitro-4-R^1-phenyl)piperidine. Heterocycles **XVIII-XXI** were obtained with yields of 89 – 97%.

This yielded a large series of condensed benzimidazole derivatives with a nodular nitrogen atom (structures I-**XX**I,

TABLE 1. Characteristics of Pyrido[1,2-*a*]benzimidazoles (**I-XVII**) and 1,2,3,4-Tetrahydropyrido[1,2-*a*]benzimidazoles (**XVIII-XXI**)

Compound	R^1	R^2	HRMS, *m/z* $[M + H]^+$	mp, °C	Yield, %
I	H	H	Calculated 169.0766. $C_{11}H_9N_2$ Found 169.0758	176 – 178 [16]	92
II	CF_3	H	Calculated 237.0639. $C_{12}H_8F_3N_2$ Found 237.0637	233 – 235 [17]	96
III	COOEt	H	Calculated 241.0977. $C_{14}H_{13}N_2O_2$ Found 241.0971	179 – 182 [17]	91
IV	CN	H	Calculated 194.0713. $C_{12}H_8N_3$ Found 194.0712	242 – 244 [17]	92
V	NO_2	H	Calculated 214.0617. $C_{11}H_8N_3O_2$ Found 214.0611	290 – 294 [18]	85
VI	Cl	H	Calculated 203.0376. $C_{11}H_8ClN_2$ Found 203.0371	212 – 214 [13]	95
VII	CN	CH_3	Calculated 222.1031. $C_{14}H_{12}N_3$ Found 222.1037	227 – 229 [17]	91
VIII	NO_2	CH_3	Calculated 242.0930. $C_{13}H_{12}N_3O_2$ Found 242.0921	214 – 217	89
IX	NH_2	H	Calculated 184.0875. $C_{11}H_{10}N_3$ Found 184.0868	178 – 182 [18]	93
X	NH_2	CH_3	Calculated 212.1188. $C_{13}H_{14}N_3$ Found 212.1183	134 – 137	91
XI	CF_3	H	Calculated 282.0492. $C_{12}H_7F_3N_3O_2$ Found 282.0485	225 – 228 [12]	92
XII	COOEt	H	Calculated 286.0830. $C_{14}H_{12}N_3O_4$ Found 286.0822	172 – 175 [12]	94
XIII	NO_2	H	Calculated 259.0469. $C_{11}H_7N_4O_4$ Found: 259.0463	276 – 278 [12]	91
XIV	NO_2	CH_3	Calculated 287.0781. $C_{13}H_{11}N_4O_4$ Found: 287.0772	195 – 199	89
XV	CF_3	H	Calculated 252.0749. $C_{12}H_9F_3N_3$ Found 252.0743	233 – 235 [19]	97
XVI	COOEt	H	Calculated 256.1087. $C_{14}H_{14}N_3O_2$ Found 256.1082	201 – 203	93
XVII	NH_2	H	Calculated 199.0984. $C_{11}H_{11}N_4$ Found 199.0981	241 – 243 [13]	91
XVIII	H	-	Calculated 173.1079. $C_{11}H_{13}N_2$ Found 173.1068	90 – 93 [11]	91
XIX	CF_3	-	Calculated 241.0953. $C_{12}H_{12}F_3N_2$ Found 241.0947	129 – 132 [11]	97
XX	CN	-	Calculated 198.1032. $C_{12}H_{12}N_3$ Found 198.1021	169 – 172 [11]	92
XXI	Cl	-	Calculated 207.0689. $C_{11}H_{12}ClN_2$ Found 207.0684	147 – 149 [11]	89

Table 1), some being compounds not previously described in the literature.

The structures of pyrido[1,2-*a*]benzimidazole derivatives (**I-XX**I) were confirmed by ^{1}H and ^{13}C NMR spectroscopy and high-resolution mass spectrometry (HRMS) (Tables 1 and 2).

Data on the antibacterial activity of substances **I-XX**I are presented in Table 3.

As shown by the data in Table 3, MIC for most pyrido-[1,2-*a*]benzimidazoles (**I-XVII**) was in the range 500 – 1000 μg/ml. Introduction of acceptor (compounds **II-V**, **XI**, **XII**) and donor (**IX**, **XVII**) substituents and substituents with different electronic natures (**XV**, **XVI**) into the benzene ring of unsubstituted heterocycle I did not in most cases increase antimicrobial activity. Only compounds containing a chlorine atom (**VI**) and two nitro groups (**XIII**) produced 100% inhibition of bacterial growth of *P. aeruginosa* and *Bacillus* sp. Respectively, at 62.5 μg/ml. The presence of two methyl

TABLE 2. ^{1}H and ^{13}C NMR Spectral Data for Pyrido[1,2-*a*]benzimidazoles (**I-XVII**) and 1,2,3,4-Tetrahydropyrido[1,2-*a*]benzimidazoles (**XVIII-XXI**)*

Compound	R^1	R^2	^{1}H NMR spectrum, δ, ppm	^{13}C NMR spectrum, δ, ppm
I	H	H	[16]	[16]
II	CF_3	H	17]	[17]
III	COOEt	H	[17]	[17]
IV	CN	H	[17]	[17]
V	NO_2	H	[18]	112.4, 113.4, 115.5, 115.8, 118.1, 128.1, 132.4, 133.3, 144.2, 146.6, 151.5
VI	Cl	H	[13]	110.8, 113.4, 116.9, 118.1, 120.5, 127.2, 127.4, 129.7, 130.8, 144.7, 148.8
VII	CN	CH_3	[17]	[17]
VIII	NO_2	CH_3	2.29 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 7.24 (s, 1H, H_3), 8.06 (dd, 1H, H_8, J 9.0, 2.1 Hz), 8.25 (d, 1H, H_9, J 9.0 Hz), 8.51 (d, 1H, H_6, J 2.2 Hz), 8.64 (s, 1H, H_1)	17.3, 18.2, 113.2, 115.5, 115.7, 121.8, 122.7, 126.7, 133.5, 143.3, 145.4, 150.8
IX	NH_2	H	[18]	100.9, 110.1, 111.6, 112.4, 116.5, 121.7, 126.8, 129.1, 146.5, 148.1, 148.3
X	NH_2	CH_3	2.27 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 5.09 (s, 2H, NH_2), 6.66 (dd, 1H, H_8, J 8.6, 2.0 Hz), 6.84 (d, 1H, H_6, J 2.0 Hz), 7.06 (s, 1H, H_3), 7.81 (d, 1H, H_9, J 8.6 Hz), 8.49 (s, 1H, H_1)	17.5, 18.2, 101.1, 111.6, 112.3, 119.2, 121.8, 122.2, 125.4, 130.4, 146.1, 147.6, 147.8
XI	CF_3	H	[12]	[12]
XII	COOEt	H	[12]	[12]
XIII	NO_2	H	[12]	[12]
XIV	NO_2	CH_3	2.32 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 7.46 (s, 1H, H_3), 8.41 (s, 1H, H_6), 8.95 (s, 1H, H_1), 9.22 (s, 1H, H_9)	17.4, 18.2, 112.1, 113.4, 115.1, 117.2, 128.1, 128.4, 133.4, 133.6, 141.6, 145.0, 152.4
XV	CF_3	H	[19]	97.0, 110.2, 112.8 q (J 6.0 Hz), 117.0 q (J 33.0 Hz), 124.1 q (CF 3, J 272 Hz), 126.2, 126.7, 129.5, 132.1, 135.3, 140.9, 147.8
XVI	COOEt	H	1.38 (t, 3H, CH_3, J 7.0 Hz), 4.36 (quin, 2H, CH_2, J 6.9 Hz), 6.40 (s, 2H, NH_2), 6.80 (t, 1H, H_2, J 6.7 Hz), 7.36 – 7.38 m (1H, H_3), 7.39 (s, 1H, H_9), 7.50 (d, 1H, H_4, J 9.3 Hz), 8.26 (s, 1H, H6), 8.60 (d, 1H, H1, J 6.7 Hz)	14.2, 60.2, 95.4, 109.9, 110.6, 117.3, 121.7, 126.8, 129.6, 133.5, 135.3, 146.2, 148.2, 167.6
XVII	NH_2	H	[13]	94.2, 101.3, 108.8, 115.8, 121.4, 125.0, 125.6, 133.0, 136.7, 138.0, 145.3
XVIII	H	-	[11]	[11]
XIX	CF_3	-	[11]	[11]
XX	CN	-	[11]	[11]
XXI	Cl	-	[11]	[11]

* Table 2 shows NMR spectral data for new condensed heterocycles (**VIII**, **X**, **XIV**, **XVI**) and previously unpublished ^{13}C NMR spectral data for some of the compounds. References to reports containing spectral data are given for other compounds.

groups in the pyridine ring of study compounds had greater influences. Thus, compounds **VII**, **VIII**, **XIII**, and **XIV** inhibited the growth of *Bacillus* sp. at significantly lower concentrations than structures **IV**, **V**, **X**, and **XI**. The antibacterial activity of 2,4-dimethyl-7,8-dinitropyrido- [1,2-*a*]benzimidazole (**XIV**) against *Bacillus* sp. was comparable with or greater than that of the efficacy of commercial tetracycline, kanamycin, levomycetin, and erythromycin formulations. Comparison of pyrido[1,2-*a*]benzimidazoles (**I**, **II**, **IV**, and **VI**) and 1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazoles (**XVIII-XXI**) showed that the latter had more marked antibacterial activity, especially against *E. coli*.

Thus, this study established that the nature of the substituent in the benzene ring had no significant effects on the antimicrobial activity of compounds. MIC was more dependent on the structure of the heterocycle annealed to the imidazole. Pyrido[1,2-*a*]benzimidazoles produced greater inhibition of the growth of Gram-positive *Bacillus cereus* bacteria. The presence of methyl groups in the pyridine fragment increased the antimicrobial action of compounds. 1,2,3,4-Tetrahydropyrido[1,2-*a*]benzimidazoles were more effective against Gram-negative *Escherichia coli* AB1157.

The mitosis-modifying and mutagenic properties of compounds **VI**, **VII**, **XIII**, **XIV**, and **XVIII**, which had the greatest antibacterial activity among the study compounds, were studied using a genotoxicity test (the *Allium* test).

Study compounds **VI**, **VII**, **XIII**, **XIV**, and **XVIII** were shown, like tetracycline and gentamicin, to decrease the number of dividing cells as compared with controls (6.1%) at all concentrations tested. Mitotic index was 5.6%, 5.7%, 3.9%, 3.1%, and 5.8% for substances **VI**, **VII**, **XIII**, **XIV**, and **XVIII** respectively. Nitrogen-containing compounds **XIII** and **XIV** were found to have the greatest effects on mitotic index. The cytotoxicity of these compounds was comparable with that of tetracycline (MI 3.3%, C = 0.01 mg/ml) and was greater than that of gentamicin (MI 6.0%, C =

TABLE 3. Antimicrobial Activity of Pyrido[1,2-*a*]benzimidazoles (**I-XVII**) and 1,2,3,4-Tetrahydropyrido[1,2-*a*]benzimidazoles (**XVIII-XXI**)

Compound	R^1	R^2	MIC, μg/ml		
			P. aeruginosa	*E. coli*	*Bacillus sp.*
I	H	H	500	500	250
II	CF_3	H	1000	1000	500
III	COOEt	H	1000	1000	500
IV	CN	H	1000	1000	500
V	NO_2	H	1000	1000	1000
VI	Cl	H	62.5	500	500
VII	CN	CH_3	500	500	62.5
VIII	NO_2	CH_3	1000	1000	250
IX	NH_2	H	1000	500	1000
X	NH_2	CH_3	500	500	500
XI	CF_3	H	500	500	500
XII	COOEt	H	500	500	500
XIII	NO_2	H	1000	1000	62.5
XIV	NO_2	CH_3	1000	500	7.8
XV	CF_3	H	1000	1000	500
XVI	COOEt	H	1000	500	500
XVII	NH_2	H	500	500	500
XVIII	H	-	500	125	500
XIX	CF_3	-	1000	500	500
XX	CN	-	500	500	250
XXI	Cl	-	500	250	500
Gentamicin			15.6	15.6	31.2
Tetracycline			15.6	7.8	7.8
Levomycetin			15.6	15.6	15.6
Erythromycin			500	500	125

TABLE 4. Phase Indexes in the Meristem of *Allium cepa* Root Shoots Treated with Study Compounds

Compound	Concentration, mg/ml	Phase indexes		
		Prophase index, % ±*m*	Metaphase index, % ±*m*	Anaphase-telophase index, % ±*m*
Control	-	56.6 ± 2.5	19.7 ± 2.1	23.7 ± 2.5
VI	0.1	48.1 ± 2.3*	28.3 ± 2.4*	23.6 ± 2.0
	0.001	50.5 ± 1.5*	26.8 ± 2.4*	22.7 ± 2.4
VII	0.01	47.4 ± 2.1*	30.3 ± 1.1*	22.3 ± 1.5
	0.001	49.2 ± 0.9*	29.6 ± 1.4*	21.2 ± 0.7
XIII	0.01	46.8 ± 0.9*	34.6 ± 0.9*	18.6 ± 0.9*
	0.001	47.5 ± 1.2*	31.2 ± 1.4*	21.3 ± 0.8
XIV	0.01	45.2 ± 1.2*	36.1 ± 2.1*	18.7 ± 0.9*
	0.001	46.7 ± 1.5*	33.6 ± 1.2*	19.7 ± 1.4*
XVIII	0.01	51.2 ± 0.8*	24.9 ± 2.0*	23.9 ± 1.3
	0.001	53.1 ± 0.7*	22.8 ± 1.2*	24.1 ± 1.2
Tetracycline	0.01	52.4 ± 1.3*	24.8 ± 2.0*	22.8 ± 2.1
	0.001	55.1 ± 1.8	21.7 ± 1.9	23.2 ± 1.4
Gentamicin	0.01	50.6 ± 1.2*	23.3 ± 1.1*	26.1 ± 1.5
	0.001	54.9 ± 1.6	18.9 ± 1.3	26.2 ± 1.5

* Significantly different from control, $p < 0.05$

TABLE 5. Frequencies of Chromosome Mutations in Cells of *Allium cepa* Root Meristem on Exposure to Study Substances (Number of Anaphase-Telophases Examined = 200)

Compound	Concentration	Chromosome aberrations, % ± *m*	Chromosome aberrations and delays, % ± *m*
Control	-	0.7 ± 0.1	1.1 ± 0.2
VI	0.01	2.1 ± 0.1*	3.1 ± 0.2*
	0.001	1.8 ± 0.3*	2.9 ± 0.2*
VII	0.01	2.4 ± 0.2*	3.5 ± 0.1*
	0.001	1.9 ± 0.2*	3.0 ± 0.1*
XIII	0.01	2.4 ± 0.1*	4.5 ± 0.1*
	0.001	1.8 ± 0.2*	3.0 ± 0.1*
XIV	0.01	4.1 ± 0.1*	5.1 ± 0.1*
	0.001	2.8 ± 0.1*	3.7 ± 0.1*
XVIII	0.01	1.4 ± 0.2*	1.7 ± 0.1*
	0.001	1.1 ± 0.1*	1.6 ± 0.2
Tetracycline	0.01	1.0 ± 0.1*	1.4 ± 0.2*
	0.001	0.7 ± 0.1	1.3 ± 0.1
Gentamicin	0.01	0.8 ± 0.2	1.3 ± 0.1
	0.001	0.7 ± 0.3	1.2 ± 0.1

* Significantly different from control, $p < 0.05$

0.01 mg/ml). Compounds **VI**, **VII**, and **XVIII** had relatively low toxicity at a concentration of 0.01 mg/ml. All study compounds produced some degree of change in the durations of mitosis phases as compared with controls, and there was an overall tendency to decreases in the numbers of cells at the prophase and anaphase-telophase stages and an increase in the numbers of cells in metaphase (Table 4).

Anaphase-telophase analysis showed that study azaheterocycles induced genetic lesions in *Allium cepa* cells (Table 5). Compound **XVIII**, containing an unsubstituted heterocycle, had the smallest effect. The largest numbers of chromosome aberration as compared with controls were obtained with compounds **XIII** and **XIV**, containing two nitro groups. At a concentration of 0.01 mg/ml, these compounds increased the number of genetic lesions by factors of 4.1 and 4.6 as compared with controls; at 0.001 mg/ml, increases were by factors of 2.7 and 3.4 respectively. The remaining study compounds displayed no significant mutagenic effects. Positive control substance tetracycline also increased mutation frequency at C = 0.001 mg/ml. Gentamicin produced no increase in the number of chromosome aberrations.

Thus, compounds **VI**, **VII**, **XIII**, **XIV**, and **XVIII** had mutagenicity and mitosis-modifying activity comparable to those of other members of this class of condensed heterocycles.

The antibacterial activity of several of the benzimidazole derivatives with nodular nitrogen atoms studied here (**VI**, **VII**, **XIII**, **XIV**, and **XVIII**), along with the low toxicity of thee azaheterocycles, leads to the conclusion that there is potential for further searches for novel antibiotics among compounds of this class.

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