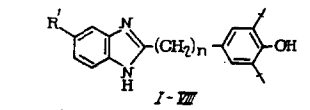
SYNTHESIS AND CYTOTOXIC ACTIVITY OF 5[6]-AMINO-2-[5′(6′)-BENZIMIDAZOLYL]-BENZIMIDAZOLES

N. Yu. Sokolova, V. A. Kuznetsov, A. V. Garabadzhiu, L. F. Ginzburg, Ya. V. Dobrynin, T. G. Nikolaeva, V. E. Fin'ko, and T. P. Ivanova

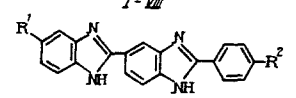
UDC 615.31:547.785.5].012.1

The biological activity of 5(6)-amino(and amido)-2-(4-R-phenyl)benzimidazoles has been reported [4]. We here present the results of a study of the cytotoxic activity of functionally substituted 5(6)-amino(and amido)-2-[5′(6′)-benzimidazolyl]benzimidazoles containing two heterocyclic fragments conjugated with each other. Continuing a study of the relationship of chemical structure to biological activity, we have examined the effects of the mutual disposition of the benzimidazole ring and a fragment containing sterically hindered phenolic groups.

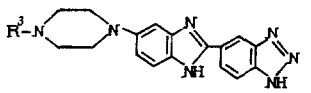
The starting materials for the synthesis of (I-VIII) were 5-R^1R^2N-1,2-diaminobenzenes [2] and the carboximidates 3,5-$(t\text{-Bu})_2$-4-$HOC_6H_2(CH_2)_n$ C(OAlk)=NH_2Cl. The laatter were obtained in the usual way [5] from the nitriles. The synthesis of the bisbenzimidazoles (IX-XI) and 5(6)-[1-(4-R-piperazyl)]-benzimidazoles containing a benzotriazole grouping at C(2) (XIII) and (XIV), has been reported [2]. Compound (XII) is the drug Hoechst 33258 from the Sigma company. Compounds (XV-XVII) were obtained as for the monobenzimidazoles (II-VIII), from the carboximidates (XVIII) [3]. The structures of the products were confirmed by their elemental analyses, UV, PMR, and mass spectra.



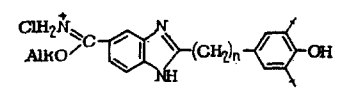
I-VIII

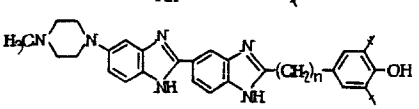


IX-XII



XIII, XIV





XV-XVII

n=0 (I), 1 (II—V), 2 (VI—VIII), XVII
R′=$N(Me_2)$, (I, II), $NH(CH_2CH_2)_2N$ (III, VI), $MeN(CH_2CH_2)_2N$ (IV, VII, XII)
R′=$CH_3CON(CH_2CH_2)_2N$ (V, VIII), $Me_2N(CH_2)_3CONH$ (IX, X),
R^3=H(XIII), Me(XIV)
R^2=ON(IX, XI, XII), NO_2(X)

Lensovet Leningrad Institute of Technology, VONTs, Academy of Medical Sciences, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 24, No. 1, pp. 31-33, January, 1990. Original article submitted May 18, 1989.

0091-150X/90/2401-0040$12.50

TABLE 1. 5(6)-Aminobenzimidazoles (II-VIII)

Comp.	Yield, %	mp, °C	Empirical formula
II	95	175	$S_{24}H_{33}N_3O$
III	90	199—201	$C_{26}H_{36}N_4O \cdot 3H_2O$
IV	89	170,5	$C_{27}H_{38}N_4O$
V	88	175	$C_{28}H_{38}N_4O_2$
VI	92	178	$C_{27}H_{38}N_4O \cdot 3h_2O$
VII	80	163	$C_{28}H_{40}N_4O$
VIII	75	175	$C_{28}H_{38}N_4O_2$

TABLE 2. Effects of 5(6)-Amino-2-[5′(6′)-benzimidazoly]benzimidazoles on Incorporation of ^{3}H-Thymidine into the DNA of Human Ovarian Carcinoma Cells, CaO_V Strain

Compound	CE_{50}	
	μg/ml	μmole/ml
I	30±5	0,164
II	22±4	0,116
III	7±1	0,028
IV	15±2	0,064
V	20±3	0,087
VI	16±2	0,032
VII	50±6	0,103
VIII	40±6	0,083
IX	60±6	0,091
X	40±4	0,062
XI	180	0,288
XII	500	0,852
XIII	200	0,536
XIV	250	0,651
XV	1,0	0,004
XVI	10±2	0,036
XVII	17±3	0,060

EXPERIMENTAL (CHEMISTRY)

UV spectra were obtained on a Shimadzu-3000 instrument (Japan) in ethanol, and PMR spectra on a Bruker AC-200 (West Germany), operating frequency 200 MHz. Mass spectra were obtained on an LKB-2091 (Sweden), ionizing electron energy 70 eV. Secondary emission mass spectra were recorded on a Varian MAT 311A (USA), xenon gas, accelerating voltage 2-6 kV, current 0.1-0.5 mA. Compounds (I-VIII) and (XIII-XV) were chromatographically homogeneous when examined by TLC on Silufol UV-254 plates in the solvent systems chloroform-methanol-25% ammonia (16:4:1) and chloroform-acetic acid-methanol (6:3:1). The elemental analyses were in agreement with the calculated values.

2-(3,5-Di-tert-butyl-4-hydroxyphenyl)-5(6)-dimethylaminobenzimidazole (I). A solution of 0.54 g (3 mmole) of 3-amino-4-nitrodimethylaminobenzene [2] in 30 ml of dioxane was hydrogenated at room temperature and atmospheric pressure in the presence of skeletal nickel. When the calculated amount of hydrogen had been taken up, the dioxane was evaporated to dryness under reduced pressure. The resulting 3,4-diaminodimethylaminobenzene and 0.99 g (2.9 mmole) of 2-methoxyethyl (3,5-di-tert-butyl-4-hydroxyphenyl)carboxymidate hydrochloride [3] were boiled in 15 ml of anhydrous acetic acid under argon for 2 h. The acetic acid was then removed under reduced pressure, and the residue dissolved in water and precipitated with 25% ammonia, and the solid filtered off and dried. Yield of (I) 0.75 g (75%), mp 225°C. UV spectrum, λ_{max}, nm (log ε) in ethanol: 257 (4.23), 3.25 (4.41). M^+ 365, $C_{23}H_{31}H_3O$.

Compounds (II-VIII) were obtained similarly (Table 1).

2-[(2-(3,5-Di-tert-butyl-4-hydroxyphenyl)-5′(6′)-benzimidazolyl]-5(6)-[1-(4-methylpiperazinyl)]benzimidazole (XV). A solution of 0.24 g (1 mmole) of 3-amino-4-nitro-[1-(4-methylpiperazinyl)benzene [2] in 30 ml of dioxane was hydrogenated over skeletal nickel (10 mass%). The catalyst was filtered off under argon, and the dioxane evaporated. To the resulting 3,4-diamino-[1-(4-methylpiperazinyl)]benzene was added a solution of 0.45 g (0.9 mmole) of 2-methoxyethyl 2-[(3,5-di-tert-butyl-4-hydroxyphenyl)]-5(6)-benzimidazolyl-carboximidate

dihydrochloride [3] in 15 ml of anhydrous acetic acid, and the mixture boiled for 3 h. It was then cooled, the acetic acid evaporated, and the residue dissolved in 50 ml of water and precipitated with 25% ammonia. The solid was filtered off and dried to give 0.36 g (75%) of (XV), mp 258-260°C (from water). UV spectrum, λ_{max}, nm (log ε) in ethanol: 272 (4.35), 342 (4.55). M^+ 536, $C_{33}H_{40}N_6O$, M 536, 721.

2-[2-(3,5-di-tert-butyl-4-hydroxybenzyl)-5′(6′)-benzimidazolyl]-5(6)-[1-(4-methylpiperazinyl)]benzimidazole (XVI). Obtained as for (XV). Yield of (XVI) 70%, mp 221-225°C, M^+ 550. $C_{34}H_{42}N_6O$, M 550, 751.

2-[2-(3,5-Di-tert-butyl-4-hydroxyphenethyl)-5′(6′)-benzimidazolyl]-5(6)-[1-(4-methylpiperazinyl)]benzimidazole (XVII). Obtained as for (XV), yield 72%, mp 205-215°C, M^+ 564. $C_{35}H_{44}N_6O$, M 564, 781.

EXPERIMENTAL (BIOLOGY)

The compounds obtained were subjected to biological tests. Cytotoxic activity was assessed in cell cultures of human ovarian sarcoma (CaO strain). The presence of activity was indicated by suppression of the incorporation of ^{3}H-thymidine into the cellular DNA.

The cells were cultured in monolayers on medium 199 with 10% cattle serum. The compounds were tested in concentrations of $5\cdot10^{-4}$, $1\cdot10^{-4}$, and $1\cdot10^{-5}$ moles/liter. The cells were exposed to the compounds for 24 h, and were then incubated for 1 h in the medium with ^{3}H-thymidine (37 mBq/ml). After washing to remove radioactivity and removal of the acid-soluble fraction from the cells, and hydrolysis with 10% $HClO_4$ at 80°C for 20 min, the nucleotides were extracted. The amount of ^{3}H incorporated was measured in ZhS-8 liquid on an Intertechnique-SL-4000 scintillation counter (France). The level of radioactivity in the samples was expressed as disint./min. For each concentration of the compound, the average percentage incorporation of ^{3}H-thymidine was calculated relative to the control, and the data used to construct plots which were used to find the half-effective doses (CE_{50}) in micrograms per ml at the 95% confidence level. The CE_{50} values were expressed in micromoles per ml.

Details of the method used have been reported [1].

The results of biological testing of (I-XVII) are shown in Table 1.

Examination of the chemical structures and biological activity of these compounds shows that the 5(6)-aminobenzimidazoles (I-VIII), which contain a sterically hindered phenolic group, all showed cytotoxic activity, the most active being those with a directly linked phenyl radical (I) or those linked via a methylene bridge (II-V). Introduction of the longer ethylene chain reduced the activity of the compounds (VII-VIII). Substituents in the 5(6)-position had little effect on cytotoxic activity, although (III), which contains the piperazine grouping, is the most active of this group.

Of the bis-benzimidazoles (IX-XII) and (XV-XVII), bearing phenyl radicals in the 2-position, (XI) and (XII) were inactive or were of low activity (IX, X), the most cytotoxic being (X) with a 4-nitrophenyl radical.

Higher cytotoxicity in nitrobenzimadazoles has been reported previously [4].

An important requirement for enhancing biological activity in bis-benzimidazoles is the attachment at C(2) of a sterically hindered phenol (XV-XVII), the most active of these compounds being (XV), in which the phenyl moiety is attached directly to the benzimidazole nucleus. Introduction of a methylene bridge (XVI) reduces cytotoxicity by an order of magnitude, further lengthening of the bridge to ethylene resulting in a further fall in activity (XVII). The structures of the substituents in the 5(6)-position have little effect on the biological activity of these compounds.

Replacement of the imidazole ring by triazole (XIII, XVI) did not confer biological activity.

LITERATURE CITED

1. Ya. V. Dobrynin, T. G. Nikolaeva, V. I. Mukhanov, et al., Khim.-farm. Zh., No. 5, 3338 (1978).
2. V. A. Kuznetsov, A. V. Garabadzhiu, and O. F. Ginzburg, Zh. Org. Khim., 23, No. 3, 637-642 (1987).
3. I. V. Sklyarova, A. V. Garabadzhiu, and O. F. Ginzburg, ibid., 22, No. 3, 645-650 (1986).
4. I. V. Sklyarova, V. A. Kuznetsov, N. Yu. Sokolova, et al., Khim.-farm. Zh., No. 6, 697-699 (1988).
5. R. Roger and D. S. Neilson, Chem. Rev., 61, 179-211 (1961).