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Influence of Some Heterocyclic, Cyclic, and Nitrogen-Containing Compounds on Oxidative Deamination of Polyamines in a Cell-Free Test System

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We studied the effects of some nitrogen-containing, heterocyclic, and cyclic compounds on the rate of oxidative deamination of polyamines and putrescine in tissues with a high proliferation rate. For this purpose, the specific activities of the main enzymes of polyamine oxidative degradation – spermine oxidase (SMO), polyamine oxidase (PAO), and diamine oxidase (DAO) were determined using a cell-free test system from regenerating rat liver. The compounds methyl 2-(5-formylfuran-2-yl)benzoate and 2,7-bis-[2-(diethylamino)ethoxy]-9H-fluoren-9-one (and in the form of dihydrochloride) showed mainly activating effect on oxidative degradation of putrescine, spermidine, and spermine, which indirectly indicates their antiproliferative effect. Nitrogen-free compounds inhibited this process, thus exhibiting potentially carcinogenic properties. Correlations were calculated for activity of DAO, PAO, and SMO with 5 topological indices: Wiener (W), Rouvray (R), Balaban (J) in the Trinaistich modification, detour (Ip), and electropy (Ie). The highest dependence was noted for DAO and the Balaban index (R=-0.55), for PAO and the detour index (R=0.78), and for SMO and the electropy index (R=0.53). The remaining dependencies showed insignificant correlation strength.

Key Words: *polyamines; diamine oxidase; polyamine oxidase; spermine oxidase; heterocyclic compounds*

Literature suggests a correlation between the level of polyamines and changes in the mitotic activity in proliferating tissues [1]. Physiological processes associated with increased cell division rate (embryonic development, regeneration) are accompanied by an increase in polyamine content [2]. Changes in cellular polyamine concentrations are also typical of some pathological conditions, including tumor growth [3]. The effect of various chemical agents on the cells proliferative activity can be assessed by the intensity of polyamine metabolism [4]. In particular, a sharp decrease in the intensity (down to zero values) of polyamine oxidative deamination by diamine oxidase (DAO) and polyamine oxidase (PAO) was revealed in mouse Gelstein's hepatomas 27, 22a, 60, 61, 46, 48, and also in primary diethylnitrosamine-induced hepatomas. The synthesis of polyamines under these conditions increased insignificantly. In contrast, the rate of polyamine synthesis in the regenerating rat liver significantly exceeds the rate of their degradation by oxidative deamination [5]. This observation allows us to assume that increased polyamine concentrations in tissues with normal and pathologically enhanced cell proliferation rate are determined by different mechanisms. Thus, it can be supposed that chemical compounds that stimulate polyamine synthesis and inhibit their degradation can have a carcinogenic effect. On the contrary, substances that inhibit polyamine synthesis and activate their degradation presumably

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may have antitumor activity. Finally, the compounds that enhance polyamine synthesis and have no effect on their oxidative deamination are likely to promote proliferation.

The use of polyamine chemical analogues is a promising trend in the development of new methods of pharmacotherapy of malignant tumors. Some of these compounds can suppress activity of atypical cells by disrupting the exchange of their own polyamines [4,6]. The emergence of new heterocyclic and nitrogen-containing organic compounds dictates the need of studying their antiproliferative effects based on the assessment of their potential influence on polyamine metabolism.

Chemical compounds that block synthesis of putrescine and polyamines and activate their oxidative degradation can act as potential antitumor agents. Chemical substances that activate polyamine synthesis and do not affect the rate of their oxidative degradation will have proliferative properties.

The purpose of the present work was to study the influence of some heterocyclic, cyclic, and nitrogen-containing compounds on the oxidative deamination of polyamines in a cell-free test system.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of the Medical Institute of the RUDN University (Protocol No. 13 of December 15, 2022) and carried out in accordance with The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Tested compounds. The studied compounds were synthesized according to standard methods [7] in the Laboratory of Synthesis of Heterocyclic Compounds of the Department of Organic Chemistry, RUDN University (Table 1). DMSO was used as a solvent for the tested substances.

Preparation of a cell-free test system. Extensive screening of the tested compounds was carried out to quantify their effects on polyamine oxidative deamination rate. White outbred male rats (n=85; body weight 250-350 g) were used in the experiments. The animals received standard diet of the RUDN University vivarium, access to water and food was free. The experimental and control groups consisted of 5 rats each. Each of the 16 tested compounds was used in one experimental group. Partial hepatectomy was performed according to the standard procedure to obtain regenerating rat liver tissue [8]. Liver tissue was mechanically homogenized in an Omni Multi-Mix200 homogenizer (Omni, Inc.). The cell-free test system was cytosolic fraction (20,000g, 20 min, 4°C, ultracentrifuge Beckman 75) of 33% liver homogenate supplemented with necessary components. The resulting supernatant was used to determine the activity of amine oxidases according to the modified method [5].

Analysis of specific activities of DAO, PAO, and SMO in a cell-free test system. To assess activities of amino oxidases, the samples of microsomal fraction of 33% homogenate of regenerating rat liver (source of amino oxidases) were incubated with one of the substrates (putrescine, spermidine, or spermine) and one of the tested compounds as described elsewhere [5]. The concentration of the tested substances in the sample was 0.1 mM. The reaction was carried out in a 96-well plate. Optical density was measured on a SpectraMax spectrophotometer (Molecular Devices). The specific activity of the enzyme was expressed in nkat per 1 mg of protein.

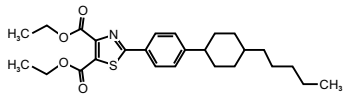
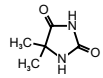
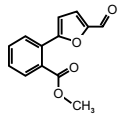
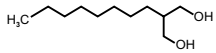
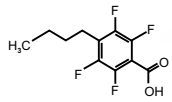
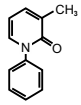
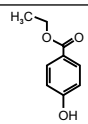
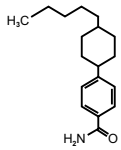
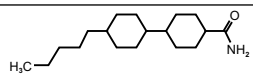
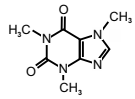
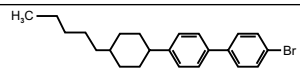
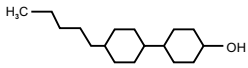
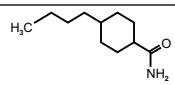
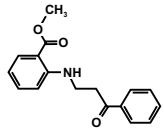
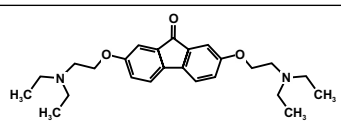
The reagents used in the experiment: horseradish peroxidase (Merck), o-dianisidine (Acros, 97%), putrescine hydrochloride (Sigma, 99.5%), spermidine hydrochloride (Sigma, 99.5%), spermine hydrochloride (Sigma, 99.5%), pyridoxal phosphate (Reanal), dithiothreitol (Serva), sodium bicarbonate (AR grade), tris(hydroxymethyl)aminomethane (Acros, 97%), potassium phosphate (AR grade), potassium hydrophosphate (AR grade; Marbiopharm), and hydrochloric acid 9.8N (AR grade).

Quantitative protein analysis. To calculate the specific activity of amine oxidases in each sample, the protein content was determined by the modified Lowry method [9].

Quantitative structure–activity relationship (QSAR). The computer programs (certificate No. 2003612305) and ChemicPen (Cetramax) were used to determine QSAR. Five topological indices were preliminarily calculated: Wiener (W), Rouvray (R), Balaban (J) in the Trinaistich modification, detour, and electropy indices according to molecular structure. Wiener index: $W=\sum D_{ii}+½\sum D_{ij}$, i>j, where D_{ii} are diagonal, and D_{ij} are non-diagonal elements of the distance matrix. Balaban index: $J=q/(\mu+1)\sum[(S_iS_j)^{-1/2}]$, where S_i and S_j are the sums of the distances of vertices i and j in the distance matrix; q is the number of connections; μ is the number of cycles. Detour index: $\omega=½\sum\sum(\Delta)_{ij}$, where Δ_{ij} is an element in the detour matrix. Rouvray index: $R=\Sigma d_{ii}+\Sigma d_{ij}$ (i>j), where d_{ii} are diagonal, and d_{ij} are non-diagonal elements of the distance matrix. Electropy index: $Ie=\log2(N!/\Pi Ni!)$, where N is the total number of atoms in the molecule; Ni is the number of atoms of the same type [10].

Statistical analysis. The obtained results were processed using Microsoft Excel 2010. The data were presented as the mean (M) and standard error of the mean (SEM). Since the data did not fit a Gaussian distribution model, nonparametric methods were used.

TABLE 1. Names, Structural Core Formulas, and Indices of New Synthesized Tested Chemical Compounds

Index	Chemical compound	Chemical formula
A1	Diethyl-2-(4-(4-pentyleyclohexypheny1)-4,5-dihydrothiazole-4,5-dicarboxylate	
A2	5,5-Dimethylimidazolidine-2,4-dione	
A3	Methyl 2-(5-formylfuran-2-yl)benzoate	
A4	2-Octylpropane-1,3-diol	
A5	4-Butyl-2,3,5,6-tetrafluorobenzoic acid	
A6	3-Methyl-1-phenylpyridin-2(1H)-one	
A7	Ethyl 4-hydroxybenzoate	
A8	4-(4-Pentylcyclohexyl)benzamide	
A9	4'-Pentyl-1,1'-bi(cyclohexyl)-4-carboxamide	
A10	1,3,7-Trimethyl-3,7-dihydro-1H-purine-2,6-dione	
A11	4-Broom-4-(4-butylcyclohexyl)-1,1-biphenyl	
A12	4'-Pentyl[1,1'-bi(cyclohexane)]-4-ol	
A13	4-Butylcyclohexane-1-carboxamide	
A14	3-Methylpyridin-2-ol	
A15	Methyl 2-[(3-oxo-3-phenylpropyl)amino]benzoate	
A16	2,7-Bis-[2-(diethylamino)ethoxy]-9H-fluoren-9-one (and as dihydrochloride)	

The reliability of the differences between the obtained data and the control values was determined by the Student's *t* test. The differences were considered statistically significant at $p \leqslant 0.05$.

RESULTS

The results of the screening of the tested compounds on a cell-free test system are presented in Table 2.

None of the studied compounds showed statistically significant inhibition of DAO. PAO activity decreased significantly only under the influence of substance A14. SMO activity decreased under the influence of agents A6, A7, A8, A9, A10, A11, A12, A13, A14, and A15. The results suggest that these compounds have carcinogenic properties.

In all other cases, mainly significant activation of DAO, PAO, and SMO was observed. The substances were ranked in the descending order of activation of catabolism of a particular polyamine, then the ranks related to the same substance, but to different polyamines were summed up. The tested compounds were arranged in decreasing order based on their ability to activate polyamine catabolism: the most active A3=A16>>A1>>>A2>A5. These results are in good agreement with the data obtained earlier in similar experiments with azafluorene derivatives [11,12].

The use of QSAR methods allows significantly decreasing the expenditure of material, labor, and time resources in the development of new drugs due to a significant reduction in the number of tested substances and a maximal narrowing of the range of side effects [13]. Successful selection of candidate substances using the ChemicDescript program is possible only in case of correct selection of the descriptor reflecting the most significant parameter for a given type of compound and for a certain activity (topology, geometry, charge distribution, *etc.*).

For activities of DAO, PAO, and SMO, correlation dependences were calculated with five specially selected topological indices: Wiener (W), Rouvray (R), Balaban (J) in the Trinaistich modification, detour (Ip), and electropy (Ie). The W index mainly reflects the size and complexity of the molecule, *i.e.* the presence of functional groups and the number of bonds and atoms, while the Balaban index takes into account the number of cycles and conjugations. The W, R, and J indices are based on the distance matrix, the Ip index is based on the detour matrix. The W and R indices are also vertex and edge-vertex graphs, because they take into account the type of atoms (vertices of the graph) and chemical bonds (edges of the graph). The electropy index indicates the diversity of atoms in the structure of a molecule.

TABLE 2. Activity of Polyamine Degradation Enzymes in a Cell-Free Test System from the Regenerating Rat Liver in the Control Group and under the Influence of the Tested Compounds (*M*±*m*)

Index	DAO, nkat/mg protein	Δ, %	PAO, nkat/mg protein	Δ, %	SMO, nkat/mg protein	Δ, %
Control	0.023±0.001	0	0.007±0.0005	0	0.006±0.0005	0
A1	0.027±0.001*	117.4	0.013±0.0004*	185.7	0.018±0.0004*	257.1
A2	0.023±0.001	100.0	0.009±0.0004*	128.6	0.018±0.0004*	257.1
A3	0.028±0.001*	121.7	0.015±0.0007*	214.3	0.02±0.0005*	285.7
A4	0.018±0.0004	78.3	0.008±0.0011	114.3	0.015±0.0005*	214.3
A5	0.022±0.001	95.7	0.009±0.0002*	128.6	0.012±0.001*	171.4
A6	0.034±0.001*	147.8	0.019±0.0004*	237.5	0.0002±0.00001*	3.8
A7	0.022±0.001	95.7	0.013±0.0007*	162.5	0.003±0.0001*	66.0
A8	0.03±0.0002*	130.4	0.01±0.0004*	125.0	0.0005±0.0001*	9.0
A9	0.035±0.001*	152.2	0.017±0.0005*	212.5	0.0002±0.00003*	3.3
A10	0.029±0.001*	126.1	0.014±0.0012*	175.0	0.0003±0.0001*	5.2
A11	0.025±0.0004*	108.7	0.014±0.0004*	175.0	0.0001±0.00001*	1.9
A12	0.029±0.0001*	126.1	0.01±0.0014*	142.9	0.004±0.0012*	78.4
A13	0.026±0.001*	113.0	0.011±0.0008*	157.1	0.0024±0.0003*	47.0
A14	0.026±0.002	113.0	0.006±0.0003*	85.7	0.00014±0.0001*	2.8
A15	0.026±0.002	113.0	0.008±0.0009	114.3	0.008±0.0001*	16.0
A16	0.03±0.001*	130.4	0.025±0.0003*	357.1	0.027±0.0005*	549.9

Note. Results are presented for 10 parallel measurements of the specific enzyme activity in each of the 5 samples. Δ,% — specific activity measured from the control, taken as 100% (>100% — activation, <100% — inhibition). *$p \leq 0.05$ in comparison with control.

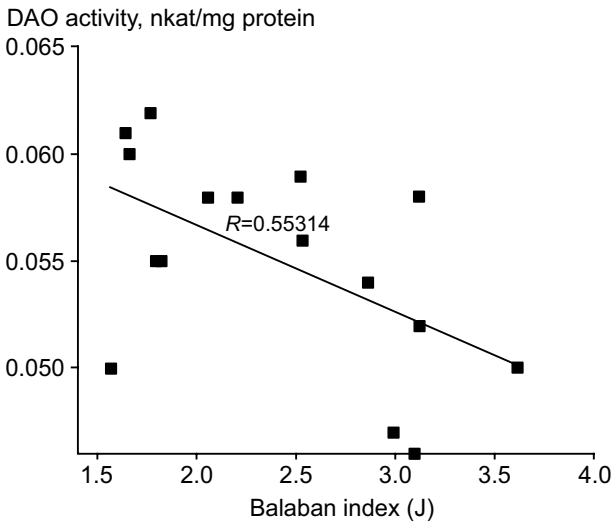


Fig. 1. Quantitative correlations of DAO activity with the Balaban index (J).

The dependence for the Balaban index and DAO activity turned out to be -0.55 (Fig. 1), for PAO and the detour index – 0.78, for SMO and the electropy index – 0.53. The remaining dependencies showed insignificant correlation strength.

Analysis of the currently available data allows us to conclude that polyamine metabolism occupies a special place in the lifecycle of actively proliferating cells, under both normal and pathological conditions. The influence of certain nitrogen-containing heterocyclic compounds on the activity of amine oxidases in liver cells was previously described, and their antitumor properties were established in L-cell lines [11]. In the present work the tested compounds were selected considering the similar results of earlier studies with derivatives of azacrown ethers, bacteriopurpurine, azafluorene, aniline, and dioxaboreninopyridine [12]. However, there have also been synthesized some similar substances for which such effects have not been studied. The products of polyamine oxidative deamination – hydrogen peroxide, iminoaldehydes, and acrolein, formed during the deamination of iminoaldehydes – are cytotoxic and can initiate apoptosis of malignant cells [14].

Further research in this direction will allow us to identify new promising antitumor agents and describe their effects on polyamine metabolism in tissues with increased proliferation rate.

The studied nitrogen-containing, heterocyclic and cyclic compounds mainly activated the catabolism of polyamines in a cell-free test system of regenerating liver, thereby demonstrating potential antitumor activity. The most active in relation to activation of amine oxidases turned out to be compounds A3=A16>>A1>>>A2>A5.

Quantitative correlations of topological indices with amine oxidase activity for the tested compounds showed the importance of the Balaban index for the analysis of this type of activity.

Conflict of interest. The authors have no conflicts of interest to declare.

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