Computer-assisted prediction of antioxidant activities and toxicities of ionol, 5-hydroxy-6-methyluracil, and their derivatives

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A mathematical model for prediction of antioxidant activity (AOA) with a recognition level of ~90% was developed using the SARD-21 computer system. Based on this model, structural modification of ionol and 5-hydroxy-6-methyluracil was carried out. A set including 32 potential antioxidants was generated. The interval levels of toxicity were theoretically predicted and the effect of structural fragments on the toxic properties of the most efficient potential antioxidants was analyzed. Structural attributes characteristic of highly efficient, low-toxicity antioxidants were revealed for the first time. Based on complex analyses of the AOA and toxicity, thirteen structures of potentially efficient low-toxicity antioxidants were proposed.

Key words: pattern recognition theory, game theory, structure—activity relationships models, molecular design, antioxidant activity, acute toxicity, ionol and 5-hydroxy-6-methyluracil derivatives.

At present, a large number of efficient natural and synthetic antioxidants (AOs) are known. However, toxicity of most synthetic AOs prevents them from being used for stabilization of cosmetics, food, and pharmaceuticals. Wide use of natural AOs is hampered by labor-consuming procedures for their extraction from natural raw materials and standardization. In this connection the development and design of highly efficient, low-toxicity AOs is topical. It is reasonable to solve this problem by combining experimental methods of investigation with theoretical ones[1] that are based on the knowledge of structure—property relationships and allow compounds with desired properties to be selected in the experimental design stage.

2,6-Di-*tert*-butyl-4-methylphenol (ionol, **I**) is widely used for stabilizing petrochemical synthesis products and as an anticancer agent.[2,3] 5-Hydroxy-6-methyluracil (oxymethyluracil, **II**) is a highly efficient immunomodulator (Fig. 1).[4] In this study, in order to design potentially efficient AOs based on commercial products, we carried out a computer experiment including modification of their structures and theoretical analysis of the toxicity level of the structures generated.

Computer Simulation Procedure

The structure—antioxidant activity (AOA) relationships were studied and molecular design was carried out using the SARD-21 (Structure Activity Relationship & Design) computer system[5,6] (a modified version of OKAS modeling system).[7] The system allows the following tasks to be solved: 1) reveal the effect of structural attributes on the manifestation of the activity type studied; 2) construct a mathematical model for prediction; 3) reveal the most promising basic structures for modification; 4) determine the priorities of the modified elements in all structures; and 5) design the target structures. Now consider the key procedures of the SARD system.[5]

Formation of the training set. The system uses a dichotomy procedure for classifying the set of compounds under study into two groups of compounds with alternative properties (*e.g.*, groups of "active" and "inactive" compounds).

Representation of the structure of a chemical compound in terms of the fragment descriptors (FDs). Three types of FDs are used: 1) initial fragments including fragments of cyclic systems and entire cyclic systems; 2) substructures comprising a number of chemically bound initial fragments; and 3) logical functions (conjunctions, disjunctions, strict disjunctions) based on the

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 8, pp. 1274—1279, August, 2006.
1066-5285/06/5508-1322

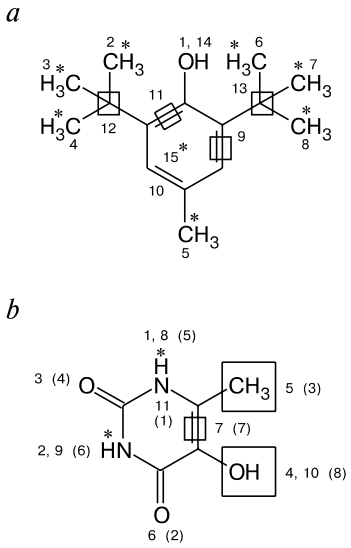


Fig. 1. Structural fragments of ionol (*a*) and 5-hydroxy-6-methyluracil (*b*) molecules tested for antioxidant activity (AOA) and toxicity. Squares denote the fragments characteristic of toxic compounds (toxophores); antitoxophores are asterisked, figures denote the numbers of fragments in the replacement matrix (Table 1), and figures in parentheses denote the priorities of replacement with respect to the AOA calculated using the minimax criterion and the replacement matrix analogously to Table 1.

first and second types of FDs. All types of FDs provide a complete description of a given structure.

Assessment of informativity of all attributes. The character of the effect of the substructure descriptors on the manifestation of activity is described[5] using the informativity coefficient (r), which varies from −1 to +1. The larger the absolute value of r the higher the probability of the effect of a given attribute on the property analyzed ("+" denotes a positive and "−" denotes a negative effect).

Construction of mathematical model for recognition and prediction. Since a complete description of groups of the compounds under study in terms of the FDs is robust, its dimensionality should be reduced and the most significant attributes (decision attribute set, DAS) should be selected. Particular attributes are included in the DAS based on the following criteria: maximum informativity, minimum interdependence, and optimal recognition of objects. The recognition and prediction models have the form of logical equations of the type $C = F(S)$, where C is the property (activity), F is the recognition rule (pattern recognition algorithm used to classify the compounds under study, namely, geometric approach or voting procedure), and S is the set of the recognizing structural parameters (DAS). The reliability of recognition of the structures investigated with respect to their AOAs approaches a value of 90%.[8]

Structure recognition and property prediction are carried out using two methods of pattern recognition theory. These are a) the geometric method based on determination of the distances to hypothetical reference structures in the classes of the active and inactive training structures and the distances to the ideal structure in the multidimensional DAS space using the Euclidean metric and b) the voting procedure based on comparison of the number of the positive and negative attributes included in the DAS and describing each structure analyzed.

Structure selection. This stage involves analysis of structural similarity between the compounds studied and hypothetical reference structures, selection of a group of basic compounds to be modified, and determination of the most favorable (from the viewpoint of activity) structural elements to be replaced and mutually interchangeable fragments. Then, the relative contributions of the elements of each structure to the targeted property are estimated and the modification routes are specified (priority of fragment replacement for each structure under study based on the game theory criteria) in order to design new structures.

Design of structures with preset properties and structure recognition using the model constructed. Hypothetical active structure are generated by replacing certain structural fragments of the basic structures by fragments typical of group of active structures following particular rules. The structures generated were assessed analogously to the classification of the training structures using the corresponding DAS.

Theoretical investigations of the toxicity of the structures generated were carried out using a prediction system[6] of acute toxicity LD_{50}, which includes a number of hierarchical prediction complexes designed for screening various classes of compounds. The corresponding toxicity prediction system was chosen with allowance for the nature of the compounds tested.

Table 1. Decision matrix for the choice of the structural fragments of **I** to be modified with respect to AOA

Initial descriptors		Informativity of fragments obtained by joining the descriptors					
Fragment number (see Fig. 1)	Type	Monad, r	Dyads, r_{max}/r_{min}	Triads, r_{max}/r_{min}	r_{max}	r_{min}	Replacement priority according to minimax criterion
1	—OH	0.571	0.688/0.385	0.559/0.097	0.668	0.097	8
2—4, 6—8	—Me	−0.088	0.316/0.316	0.314/0.300	0.316	−0.088	2
5	—Me	−0.088	0.097/−0.014	0.097/0.036	0.097	−0.088	1
9—11	>C=C<	0.243	0.331/0.241	0.559/0.036	0.559	−0.041	5
12, 13	>C<	0.125	0.316/0.241	0.314/0.097	0.316	0.097	3
14	H (Het)	0.652	0.571/0.571	0.668/0.385	0.668	0.385	9
15	Ar*	0.385	0.385/0.097	0.301/0.097	0.385	0.097	4

* 1,2,3,5-Tetrasubstituted aromatic fragment.

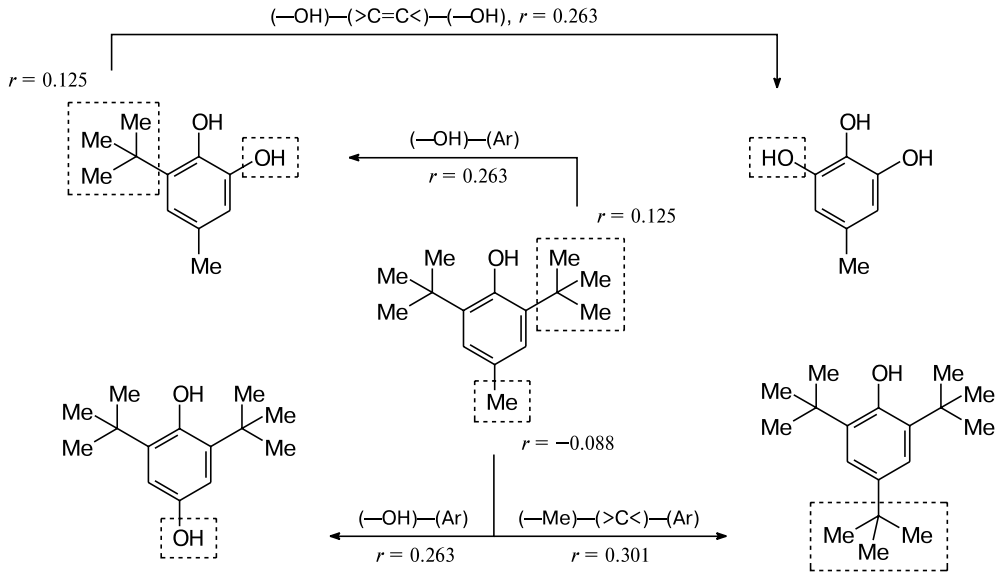


Fig. 2. Structural modification of ionol molecule **I**; *r* is the informativity coefficient; dashed lines frame the molecular fragments to be modified and the results of modification.

To reveal functional groups characteristic of efficient low-toxicity AOs, we performed a complex analysis of structural attributes of two models of the AOA and toxicity.

Results and Discussion

The structures of the compounds under study were modified in accordance with the Scheme shown in Fig. 2 with allowance for the priorities of replacement of the structural fragments (see Table 1 and Fig. 1). For instance, the antioxidant properties of compound **I** are almost not associated with the Me group in position 4 of the benzene ring and the Me groups bonded to the quaternary carbon atoms in the side chain (see Table 1). Therefore, these groups should first of all be replaced. At the same time these fragments are characteristic of low-toxicity compounds (see Fig. 1). Since there is a large number of Me groups in molecule **I**, replacement of one of them should not cause an increase in toxicity of the compounds designed.

Using the procedure described above, a set comprising twenty-six structures of potentially efficient AOs **1—26** based on **I** was generated (the known AOs are asterisked).

Among them, seventeen structures correspond to the known compounds possessing the properties of AOs (*e.g.*, epigallocathechin **26**, which is a natural AO). Table 2 lists the kinetic characteristics of AOA (rate constant for the reactions with peroxy radicals) for some of these compounds.[10] The AO properties of the molecules designed confirm the correctness of the structure—AOA relationships proposed and the possibility of using them in the molecular design of organic compounds.

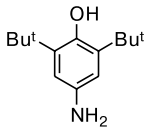
Table 2. Kinetic characteristics of antioxidants

Structure	Solvent	*T*/K	$k_{in} \cdot 10^{-4}$ * /L mol^{-1} s^{-1}	Reference
2	Polystyrene	338	8.30	9
3	Ethylbenzene	338	15.50	10
6	Styrene	338	0.50	9
13	Styrene	338	4.17	10
18	Ethylbenzene	333	1.32	10
19	Ethylbenzene	333	4.9	10

* Rate constant for the reaction of inhibitor with peroxy radicals in solvent.

We also designed a set of thirty-two structures based on structure **II**. Six of them (structures **27—32**) can be considered as are potentially active AOs.

Complex analysis of the structural attributes in the framework of the AOA recognition model using the toxicity prediction system showed that amino group, fragments containing 1,2,3,5-tetrasubstituted aromatic fragment, and combinations of the methyne fragment with the double bond *via* the oxygen atom (Figs. 3 and 4) belong to active, but rather toxic molecular fragments. The group of potential toxophores includes all sulfur-containing fragments (see Fig. 3). One can expect that the antioxidant properties of the compounds will be favored by such toxophores, as (>C=C<)—(>C<)—(>C=C), (—Me)—(>C=C<)—(>C=C<), (—Me)—(—O—)—(Ar) (Ar stands for 1,2-disubstituted benzene), (>CH—)—(>CH—)—(—NH—), and a combination of amino group with azomethyne fragment. The secondary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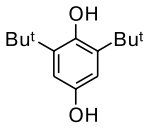
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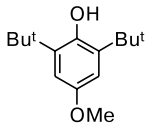
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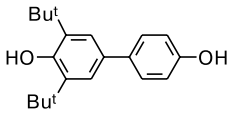
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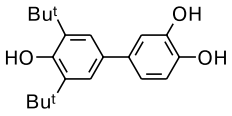
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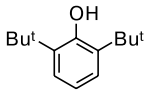
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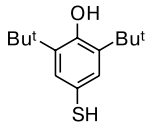
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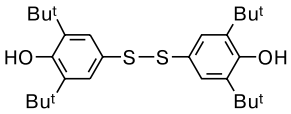
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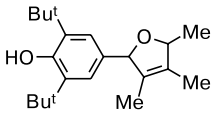
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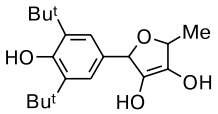
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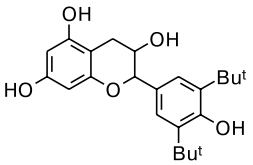
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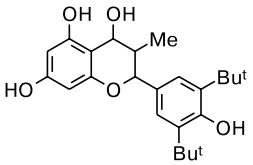
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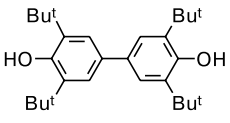
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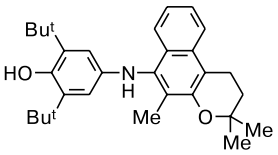
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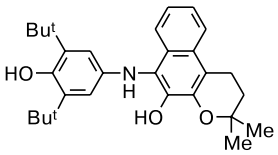
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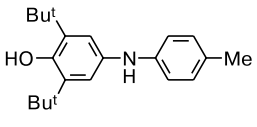
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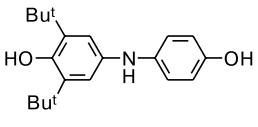
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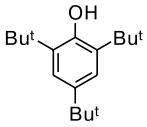
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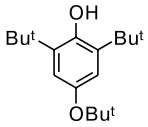
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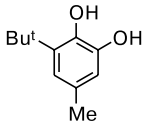
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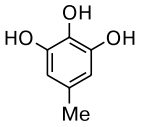
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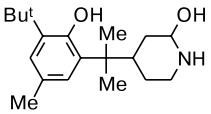
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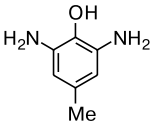
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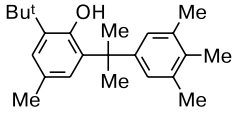
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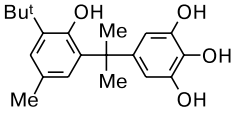
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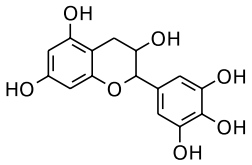
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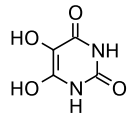
amino group, both as individual attribute and as a constituent of fragments containing the quaternary carbon atom and 1,4-disubstituted aromatic moiety or azomethyne fragment, acts as an antitoxophore characteristic of efficient AOs because of small *r* value with respect to toxicity.

The attributes that enhance the AOA and reduce the toxicity include a combination of a >CH— unit with a secondary amino group and a hydrogen atom bonded to the heteroatom (see Fig. 3, *a*, *c*). A similar effect is expected from structural fragments containing a carbonyl group bonded to hydroxyl group. Fragments character-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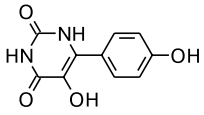
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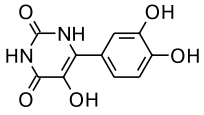
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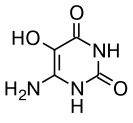
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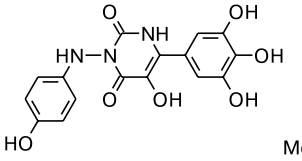
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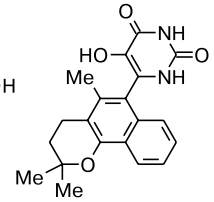
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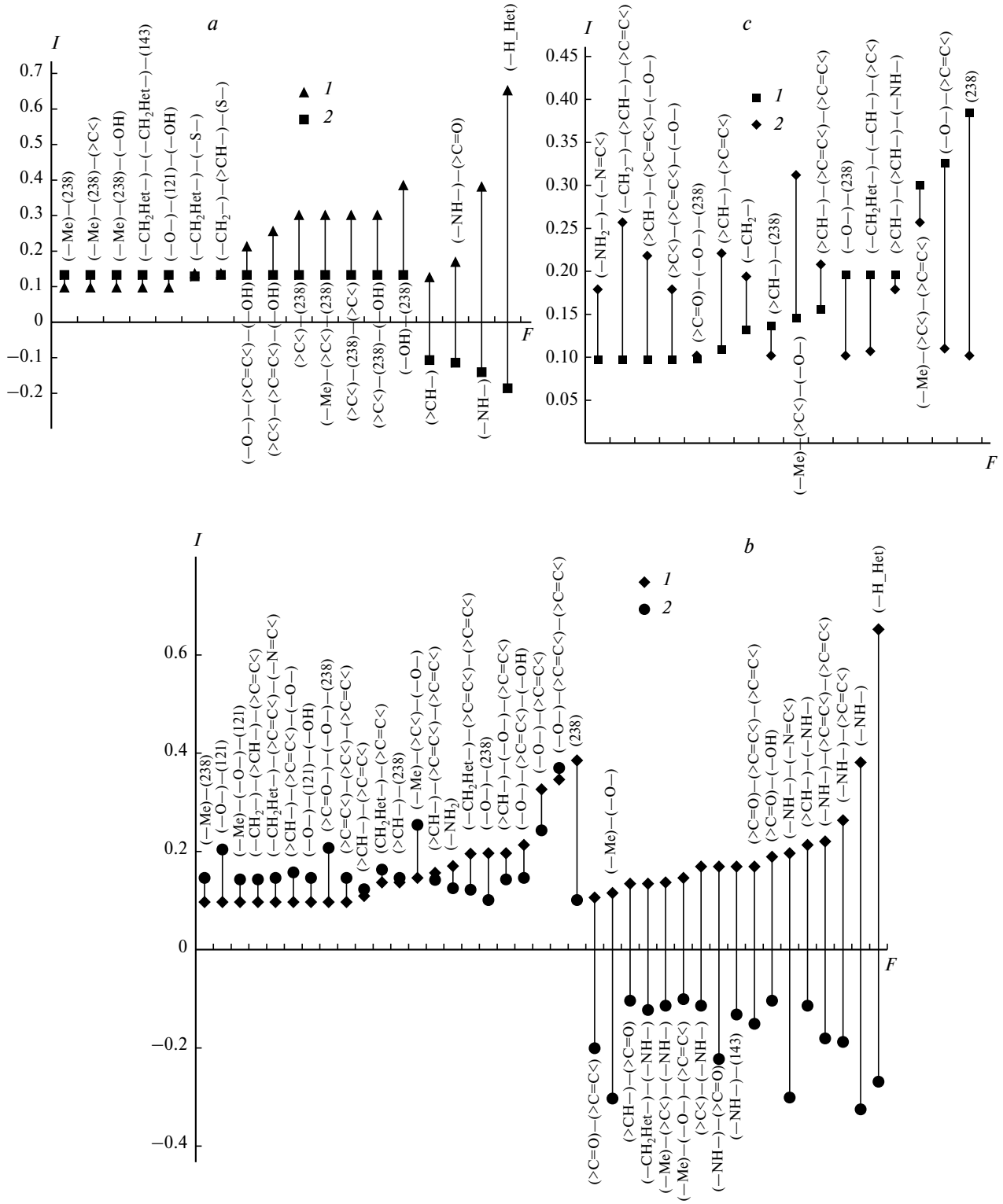

Fig. 3. Effect of structural attributes on the antioxidant activity (*1*) and toxicity (*2*) of nitrogen-, sulfur-, and oxygen-containing heterocyclic compounds (*a*), saturated and unsaturated nitrogen-containing compounds (*b*), and oxy derivatives of aromatic heterocyclic carboxylic acids (*c*); *I* stands for informativity.

ized by high *r* values with respect to AOA, obtained as a result of aggregation of the secondary amino group with one or more double bonds in cyclic systems can also favor the inhibition of toxic properties (see Fig. 3, *a*, *c*).

Theoretical tests of the structures generated for the presence of toxic fragments revealed that in molecule **I** the most hazardous (from the viewpoint of toxicity) fragments combine the quaternary carbon atom and ethylene groups of the ring (see Fig. 1). The Me group at the ring and the Me groups bonded to the quaternary carbon atom are expected to be relatively less toxic in this compound. In molecule **II**, potential toxophores include the double bond and the neighboring Me and OH groups (see Fig. 3, *b*). The hydrogen atom at the heteroatom, as well as the carbonyl group—double bond combination (see Fig. 3), which are characterized by low *r* value with respect to toxicity, are expected to reduce the toxicity of the compound.

The organic compounds, which possess the properties of antioxidants and are at the same time potential pharmaceuticals, were checked for toxicity. Based on the results obtained, compound **I** and its derivatives can be treated as medium-toxicity substances. One can expect a relatively higher level of toxicity (~150 mg kg^{-1}) for structures **11** and **26**. In structure **11**, the undesired and decisive (from the viewpoint of toxicity) is the combination of fully substituted carbon atom and the ethylene and OH groups. The toxicity of the structures **1**, **3**, **19**, and **23** is expected to lie between 150 and 600 mg kg^{-1}. The toxicity of most structures (**I**, **2**, **4—8**, **13—15**, **18**, **20—22**, **24**, **25**) is predicted to be in the range 600—1000 mg kg^{-1}, except structures **9** and **10**, for which it spans the interval from 600 to 1500 mg kg^{-1}.

Complex analysis of the AOA and toxicity of the derivatives of compound **II** in the framework of the models used revealed a low level of toxicity for all six potentially efficient compounds **27—32**. According to the amide toxicity model, the toxicity of compound **II** exceeds a value of 5000 mg kg^{-1} and those of its derivatives **27** and **29—31** are in the range 600—5000 mg kg^{-1}. Estimates of the interval levels of toxicity made for compounds **28** (3000—5000 mg kg^{-1}) and **32** (1500—5000 mg kg^{-1}) allow them to be classified into low-toxicity compounds. These results are in good agreement with the published data. For instance, compounds **I** and **2** are characterized by medium level of toxicity (LD_{50}(**I**) = 1700—1970 mg kg^{-1}; LD_{50}(**2**) = 2000—2200 mg kg^{-1}; oral administration).[3] Experiments with white male rats revealed a high toxicity of compound **II**, its lithium salt, and 6-methyluracil upon single-dose oral administration (LD_{50}(**II**) > 6000 mg kg^{-1}).[4] The preparations studied exhibit no cumulative effect, namely, the LD accumulation index for **II** is 18.7% (experiments with mice) and the deactivation rate of **II** in male mice is 116.6 mg h^{-1} kg^{-1}.

Thus, the synthesis and investigation of the properties of compounds **9**, **10**, **14**, **15**, **22—25**, **28**, **29**, **31**, and **32** obtained by structural modification of molecules **I** and **II** seems promising. Calculations predict a medium or low toxicity and efficient antioxidant properties of these compounds.

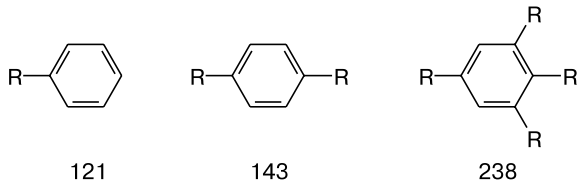

Fig. 4. Cyclic fragments found by joint analysis of the attribute spaces of the models for prediction of the AOA and acute toxicity; shown are the ring codes used in the calculations.

The results obtained in this work show that mathematical models for prediction of the AOA and the interval levels of toxicity can be used for rapid evaluation of the antioxidant and toxic properties of organic compounds. By applying these models to the structures of the known AOs one can predict the structures of new potentially nontoxic inhibitors of oxidation processes.

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Received August 3, 2005;
in revised form June 9, 2006