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Quantum-chemical Calculations in the Study of Antitumour Compounds

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The results of quantum-chemical calculations on antitumour preparations concerning the mechanism of their action at the electronic and molecular levels and structure-activity correlations are discussed in this review. Preparations whose action involves alkylating and free-radial mechanisms, complex-forming agents, and antimetabolites are considered. Modern quantum-chemical methods for calculations on biologically active substances are described.
The bibliography includes 106 references.

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1. INTRODUCTION

The wide-scale employment of the ideas and methods of chemical kinetics and physical organic chemistry in experimental and clinical oncology have led to the creation of a new field of fundamental and applied research—the physical chemistry of cancer, founded by Academician N.M.Emanuel'.[1,2] In relation to the current problems of tumour chemotherapy, these approaches have been directed towards the study of the relation between the structure and chemical reactivity of antitumour compounds (ATC) on the one hand, and their biological effects on the other. ATC are distinguished from other types of medicinal preparations by including representatives from different classes of chemical compounds. For this reason, the antitumour effect of the preparations at the molecular level cannot be accounted for in terms of a single mechanism of their action.

Fundamental principles governing the creation of new effective antitumour preparations, which have been developed by Emanuel', include the classification of ATC in terms of their chemical reactivities.[3,4] This classification constitutes the basis of the study of the anticancer preparations at the electronic and molecular levels and the establishment of quantitative structure—activity correlations, in which quantum-chemical calculations play an important role.[4]

Modern quantum-chemical methods are widely used in studies on biomacromolecules and biologically active compounds (BAC). Recently ATC have also begun to be actively investigated by these methods. Whereas in 1976 the lack of information about calculations for ATC was noted in Pullman's monograph[5] on quantum-chemical calculations of the conformations of medicinal preparations, by 1984 several tens of articles devoted to quantum-chemical studies on preparations of this kind were already published in the world scientific

literature. This review contains information about quantum-chemical calculations of the electronic parameters of ATC molecules and about the modelling of the mechanisms of their biological action at the electronic and molecular levels.

II. CHARACTERISTIC FEATURES OF THE USE OF QUANTUM-CHEMICAL METHODS IN THE STUDY OF BIOLOGICALLY ACTIVE COMPOUNDS

1. Quantum-chemical Calculations of the Electronic Structure

According to the postulates of quantum mechanics, the structure and properties of the molecule can be determined from its wave function Φ. The wave function is found by solving the fundamental quantum-mechanical equation—the Schrödinger equation. As a result of the development of computer techniques, fairly accurate methods have been devised in modern quantum chemistry whereby the Schrödinger equations can be solved for the stationary states of many-electron and polyatomic systems to which BAC belong. Quantum-chemical methods in which Φ is formulated as an antisymmetrised product of the spin orbitals whose coordinate components (MO) are represented by linear combinations of atomic orbitals (LCAO) have come to be most widely employed in studies on BAC. The positions of the nuclei are assumed to be frozen (adiabatic approximation). The quantum-mechanical calculation reduces to finding the coefficients of the expansion of the MO in terms of the AO by solving the Hartree–Fock–Roothaan equations, which consist of a system of algebraic equations. Several monographs on the principles of quantum chemistry have now been published[6-9] and a more detailed exposition of questions concerning the computational methods, which are only briefly described here, may be found in them.

The quantum-chemical calculation yield information about the spatial distribution of electrons in the molecule and about the energy levels of the electrons. The total energy of the system includes contributions by the one-electron orbital energies and the integrals of the Coulombic and exchange interactions. Despite the approximations underlying it (in particular the neglect of the correlation energies of electrons), the application of the Hartree–Fock–Roothaan method makes it possible to estimate fairly accurately many phyisochemical characteristics of many-electron molecules. Depending on the approaches to the solution of the Hartree–Fock–Roothaan equations used, the quantum-chemical methods are divided into non-empirical (*ab initio*) and semiempirical.

The non-empirical methods are based on a rigorous solution of the Hartree–Fock–Roothaan equations. In the calculations account is taken of all the electrons in the molecule and of all the interelectron interaction integrals. An important feature of the *ab initio* calculations is the choice of the form of the AO basis set in terms of which the MO are expanded. Various representations of the AO in the form of Gaussian functions are used nowadays for calculations on polyatomic molecules. The total energy in non-empirical calculations using different selections of the AO basis set varies within the limits of several per cent relative to its lower limit attainable by the one-determinant Hartree–Fock method.[8] However, the accuracy of the calculation of the physical properties depends very greatly on the choice of the AO basis set. It has been noted in a review[10] that, for a correct calculation of the molecular geometry and the orbital energies (for example, for comparison with photoelectron spectra), it is sufficient to employ a minimal basis set; the calculation of the isomerisation energies and conformational properties requires the use of a two-exponent basis set; for the calculation on negatively charged ions and excited states, it is essential to employ a two-exponent basis set with allowance for diffuse orbitals; the exact calculation for van der Waals interactions, spin densities, and magnetic permeabilities makes it necessary to abandon the one-determinant approximation and to employ the multiconfiguration Hartree–Fock method with a basis set of two-exponent and polarisation functions.

The increase in the complexity of the sets of basis functions entails a sharp decrease in the molecular size susceptible to calculation, which is due to the increase in computing time required to evaluate the electronic integrals and to the limited capacity of the operating memory and the peripheral memory devices of the computer. For this reason the simplest possible basis sets are used for calculations on BAC,[11] the minimal STO-3G basis set being widely employed. Non-empirical calculations for a wide range of organic compounds in terms of this basis set reliably reproduced the equilibrium geometry of neutral molecules, the heats of atomisation, the dipole moments, and the molecular electrostatic potentials.

Much attention has been devoted recently to the development of *ab initio* methods disigned for the direct investigation of large molecules.[12] The computational aspects of the non-empirical calculations have in this instance certain unusual features; in particular, owing to the large interatomic distances within the molecule, there is a possibility of an appreciable diminution of the number of two-electron integrals differing appreciably from zero. It has been suggested that the number of integrals considered be reduced by a rigorous consideration of only the valence electrons and the employment of a model potential (MODPOT) for the electrons of the skeleton,[13] by variable [partial] retention of [diatomic] differential overlap (PRDDO;[14] VRDDO [15]), and by the ruse of the integrals for similar strucual fragments (MERGE).[16] It has been noted that, when the very latest CRAY computers with a vector processor are used, the integrals are calculated so rapidly that it becomes reasonable to recalculate them anew each time instead of reading them from a peripheral memory device.[17] In this case the computer memory stores several sets of indices of non-zero integrals. Such "approximate" non-empirical methods, whose accuracy differs little from that of the calculations in terms of the STO-3G basis set, permit an appreciable saving in computing time—VRDDO by 25%, VRDDO/MODPOT by a factor of 3, and PRDDO by a factor of 15 compared with STO-3G.[18] The gain in time increases with increase of the number of AO. The employment of the PRDDO and VRDDO methods is apparently optimal (in relation to the accuracy/computing time ratio) in calculations on BAC.[18]

Despite the endeavour to employ rigorous non-empirical methods, various approximate methods for finding the wave functions, referred to as semiempirical methods, arose in the course of the development of quantum chemistry. Their appearance is associated with the methodological and technical difficulties which occurred and still occur in rigorous *ab initio* calculations. In the semiempirical methods the Hartree–Fock–Roothaan equations are solved subject to a series of approximations in the formulation of the Hamiltonian, which are based on the allowance for the interaction of only some of the electrons of the molecule (electrons or valence electrons) and the neglect of some of the inter-electron repulsion integrals. The introduction of such approximations is compensated by employing, in constructing the Hamiltonian, semiempirical parameters selected on the basis of experimental data. By means of a correct choice of parameters in the semiempirical method, it is possible to reproduce correctly in qualitative terms certain physicochemical properties of the molecules and, in particular, their variation in series of related compounds. However, as a result of the loss of rigour in the treatment, certain molecular characteristics and

intermolecular interaction energies reproduced by the semiempirical methods are in principle incorrect. Therefore the use of semiempirical calculations makes it necessary to estimate in each case the scope of the approach selected for the solution of the given problem.

Computer programmes now exist for a whole series of different quantum-chemical methods which form a virtually continuous spectrum between simple schemes with complete neglect of differential overlap (CNDO) and rigorous *ab initio* approaches. The following approaches are employed most frequently in quantum biochemistry: different versions of the Hückel method, methods of the CNDO type (CNDO/2, CNDO/BW, CNDO/S), INDO, INDO/S, MINDO/2, MINDO/3, MNDO, VRDDO, and the *ab initio* STO-3G method. In the above list the methods are arranged in order of increasing rigour of the treatment of the Hartree–Fock–Roothaan equations. The practical choice of the method is usually dictated by its feasibility as regards its application on a given computer and by the character of the problem. In the USSR there is a library of quantum-chemical programmes (analogous to the library of QCPE computer programmes available in the USA),[19] which apply the majority of standard semiempirical and non-empirical methods. The programmes are designed for computers of the BESM-6 type, which remain the most widely used in scientific centres, and EC. A detailed comparison of the characteristics of Soviet and foreign computers and their scope as regards the solution of quantum-chemical problems may be found in the book of Shchembelov et al.[9]

2. Analysis of the Reactivities of BAC

In the first stage of a quantum-chemical study, one selects and determines the molecular structure of the test compound. Data on molecular structure can be obtained experimentally (by the X-ray diffraction and spectroscopic methods and by gas electron diffraction). In many cases such structural information is subsequently used directly to calculate the wave function. In those cases where experimental data are lacking or are incomplete, the equilibrium molecular structure is calculated by a quantum-chemical method. For this purpose, the total energy of the molecule, which is a function of the structural parameters (the atomic coordinates or the generalised coordinates), is minimised with respect to these parameters. The use of gradient optimisation methods in combination with an adequate choice of the methods for the calculation of the wave function itself, usually gives rise to a satisfactory quantitative agreement with experiment.[8] The conformational analysis of the BAC also constitutes a separate problem, which is important for the elucidation of the specific features of their interaction with receptors and for the synthesis of new medicinal drugs.[20]

The reactivities of BAC are usually analysed by considering the reactivity indices (RI). These are defined as the molecular characteristics, calculated quantum-chemically, from which one can infer the occurrence of a specific chemical reaction. A simple physical characteristic of the molecule can serve as an index of this kind. The expected value of such a physical property M is calculated, in conformity with the postulates of quantum chemistry, by the formula

$$M = \int \Phi \hat{M} \Phi^* dV ,$$

where $\hat{M}$ is the corresponding M operator and Φ^* is the complex conjugate of the wavefunction Φ, and the integration is carried out over all the variables in Φ. The characteristics which depend solely on the properties of the isolated molecule determine the changes in the electronic structure of the molecule at an early stage of the reaction and they are referred to as the static RI. The parameters which take into account the mutual influence of the reactants describe the properties of the molecule at an intermediate stage of the reaction and are referred to as the dynamic RI. The RI permit only a relative conclusion, i.e. they make it possible to infer the variation of reactivity in a series of related compounds. The main condition governing the applicability of the RI is that the principle of the linear variation of the free energies holds for the given series at all stages of the reaction, which means that, when the energies of the initial reaction stage for a series of n compounds are arranged in the sequence $E_1E_2...E_n$, then this same sequence should hold also subsequently.

The principal RI characterising the energetic and structural parameters of molecules as well as the corresponding physicochemical (including spectroscopic) properties of the molecules are presented below (Fig.1).

The majority of the indices quoted are static. The significance of a particular index in each case is determined by the nature of the process investigated. In the case of a bimolecular reaction the overall expression for the energy of the interaction of the reactants E_t can be obtained from perturbation theory:[8]

$$E_t = E_Q + E_{ind} + E_{ex} + E_{ct} + E_{disp} ,$$

where E_Q is the energy of the Coulombic interaction, E_{ind} the energy of the inductive interaction, E_{ex} the energy of the exchange interaction, E_{ct} the energy of the charge transfer interaction, and E_{disp} the energy of the dispersion interaction. The total energy E_t is itself the most general dynamic RI. The formulae for the different contributions to E_t have been published.[8] When the reaction depends at some stage mainly on interactions of one kind, the expression which can be fitted to the corresponding interaction may serve as a satisfactory dynamic index. In such cases it is frequently possible to isolate also the static RI on the basis of which one can predict the reactivity in the series of substances investigated. For example, electrostatic interactions play an important role in the formation of hydrogen-bonded complexes and the corresponding index, the molecular electrostatic potential (MEP), is satisfactorily correlated with the total energy of the hydrogen bond.

The quantum-chemical analysis of the reactivities of BAC is nowadays performed in most cases for isolated molecules. Such models correspond to gas-phase reactions and the feasibility of transferring the results of calculations for such reactions to liquid-phase biological processes requires additional justification. According the Reichardt[21] the solvent exerts a stronger influence on the reactions in which the transition state of the molecule has a dipolar structure. The polarity of the solvent is then of decisive importance. In cases where the transition state is not characterised by an isopolar or free-radical structure, the influence of the solvent is small. In calculations of reactivities in such cases one can apparently restrict the treatment to the approximation of an isolated molecule. The solvent influences the conformational characteristics and the reactivity both via its macroscopic properties (dielectric constant, surface tension) and via specific solvation in which the strong interaction (hydrogen bonding, the formation of a π-complex) between the solvent and solute molecules is important. These effects are taken into account in quantum-chemical calculations within the framework of "continuous" models and "supermolecular" models,[22] but the methodological difficulties associated with allowance for solvation effects remain one of the problems in quantum chemistry.

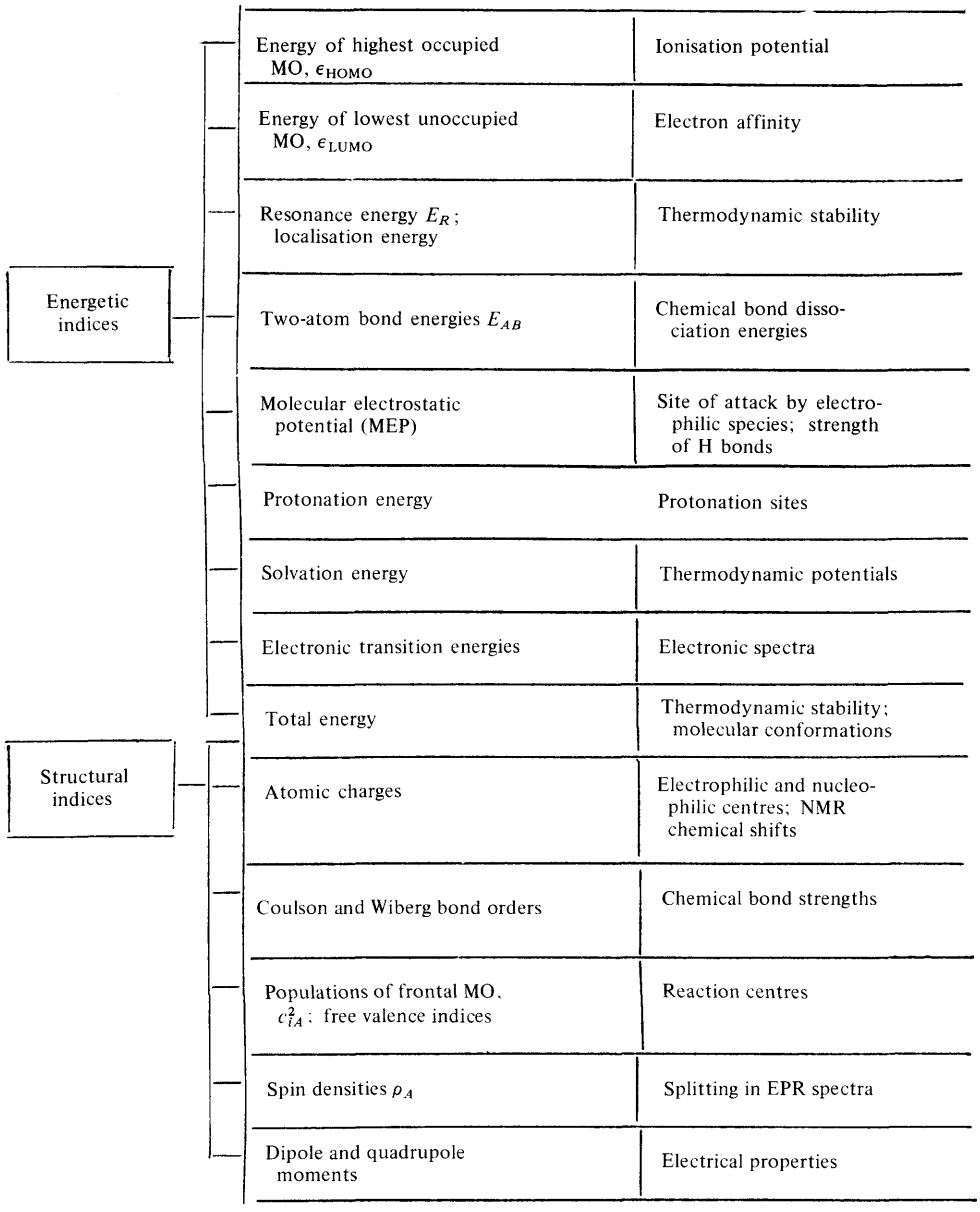


Figure 1. The reactivity indices used in the study of BAC and their physicochemical interpretation.

3. Statistical Structure–Activity Relations

The parameters of the electronic structure carry direct information only about chemical reactivity. In order to reach conclusions of a biological character, it is necessary to trace the relation between the RI (chemical interaction) and the biological effect of the compound. Quantum-chemical calculations sometimes make it possible to put forward a hypothetical model of the biological activity of just one preparation on the basis of the analysis of experimental data for its biological activity. However, it is more usual to calculate the electronic parameters for a series of compounds with similar structures for which the quantitative characteristics of the biological effects investigated are known, and then the question of the existence and nature of quantitative structure–activity correlations (QSAC) is investigated.[23,24] QSAC are usually analysed in terms of Hansch's model,[25] in which the rate of the biological response k_{br} is represented in the form

$$k_{br} = Ack_r,$$

where A is the probability that the BAC molecule reaches the receptor, c the extracellular concentration of the BAC, and k_r the rate of reaction between the BAC and the receptor. The biological effectiveness is determined by the concentration c necessary to attain a specified level $k_{br} = \text{const}$.

A Gaussian relation governing the lipophilicity coefficient π, namely

$$\pi = \lg P_s - \lg P_0 ,$$

where P_S and P_0 are the partition coefficients between water and octanol (octanol is used as the model of a lipid) of the given (s) and initial (0) compounds, is used for the quantity A characterising the transition from the aqueous phase via the lipid membrane into the cell. The rate k_r is determined from the Hammett equation $k_r = \rho\sigma$. The final equation relating c to the parameters of the compound is

$$\lg(1/c) = K_1\pi - K_2\pi^2 + \rho\sigma + K_3 , \quad (1)$$

where K_1, K_2, and K_3 are constants found by regression analysis. In many cases it is possible to restrict the treatment to the consideration of only the linear terms of Hansch's equation. Such regression relations are also used with parameters of other types, for examples, those of the Free–Wilson and Cammarata models.[26–28] None of these models presuppose quantum-chemical calculations. However, it is now recognised that quantum-chemical RI offer greater possibilities for the analysis of structure–activity relations.[23,24]

A direct relation has been traced between the hypotheses underlying Hansch's equation and the condition governing the applicability of the RI. In each case it is then as a rule possible to select quantum-chemical parameters which are more effectively correlated with the k_r than the Hammett constants. This has been responsible for the wide-scale application of RI in deriving relations of type (1). Numerous different simple static RI are used nowadays in QSAC. This hinders regression analysis and yields little information about the mechanism of the action of the preparation. It has been suggested[29] that statistically independent linear combinations of RI (principal components) be employed. Such an approach simplifies the statistical treatment but makes it difficult to interpret the mechanism of the action of the given compound. In our view the reliability of QSAC should be improved by employing complex dynamic RI which are more effectively correlated with the k_r.

The transport properties of chemotherapeutic preparations, which depend both on the structural-functional characteristics of the ATC (molecular size, pK, and lipophilicity) and on the rate of blood flow, the permeability of cytoplasmic membranes, the rate of biotransformation etc., are important for the manifestation of the antitumour activity of such preparations.[30] The transport of chemicals across biological membranes diminishes with increase of the degree of ionisation and the molecular weight passes through an extremum (as a rule a maximum) as a function of the lipophilicity parameter π.[31] In view of its complexity, the real picture of the pharmacokinetic behaviour of the ATC in the organism and in the cell is very difficult to calculate theoretically. Hansch's equation reflects only the dependence of the biological response on the transport of the ATC across the membrane, which is in its turn specified as a function of the hydrophobic properties alone.

The experimental values of π are as a rule used in QSAC. Theoretical estimates of π are usually obtained with the aid of empirical additive schemes for series of related compounds.[32,33] The hydrophobic properties of the preparation can be inferred approximately also from quantum-chemical calculations for intermolecular interactions. A direct calculation of π from the thermodynamic characteristics of the solute, evaluated by the CNDO/2 method, has been carried out for the conformers of prostaglandins.[34] A method has been proposed[35] for estimating π from the charge distribution and the hyperconjugation indices of the isolated molecule. The fact that, according to thermodynamic equations, π is proportional to the difference between the free energies of the substance dissolved in water and in the lipid phase can also be used in estimating the lipophilicity parameter. Qualitative agreement between the behaviour of π and the hydration energies calculated in the electrostatic approximation using the MEP on the van der Waals surface of the molecule has been achieved[36] for a series of sterically hindered phenols.

Thus information about the electronic and molecular mechanisms of the action of the preparation can be obtained and new chemical structures with a potentially high activity can be synthesised on the basis of "quantum-chemical parameters–activity" correlations. Questions concerning the strategy of the employment of quantum-chemical calculations in the study of BAC were described previously in a series of reviews.[23,24,37] Detailed information about the mathematical methods whereby one can seek the relation between biological activity and chemical reactivity has been published.[38]

III. QUANTUM-CHEMICAL CALCULATIONS AND THE CHEMICAL MECHANISMS OF THE ACTION OF ANTITUMOUR PREPARATIONS OF DIFFERENT CLASSES

Antitumour preparations have come to be divided into classes as a function of their chemical nature, their origin, or the mechanism of their action. The classification[39] in which the ATC are divided into (1) alkylating agents, (2) antimetabolites, (3) various synthetic preparations, (4) medicinal drugs of natural origin (antibiotics, plant preparations, enzymes), and (5) hormonal preparations is widely used.

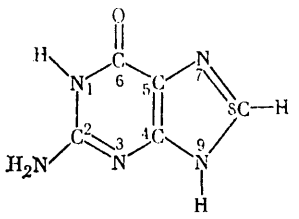
The classification of antitumour preparations in terms of the principle of chemical reactivity has been proposed by Emanuel'.[3,4] In this classification the ATC are divided into biochemically functionally active (analogues of compounds involved in cell metabolism) and chemically functionally active (characterised by a purely chemical interaction with cell molecules). The chemically functionally active ATC known at the present time are in their turn divided into groups in accordance with the chemical reactions which determine their antitumour activity. Here one encounters three main types of reactions: electrophilic reactions, complex formation, and free radical reactions. ATC with a combined mechanism of their action apparently also exist. In view of the latter factor, certain preparations, for example, the antitumour anthracycline antibiotics and sterically hindered phenols, cannot be at present assigned to a specific class. However, the classification in terms of the types of reactivity is more logical than others and can serve as a basis for the purposeful search for chemical compounds with a high antitumour activity. The results of quantum-chemical calculations on the ATC are presented below in conformity with their subdivision on the basis of their chemical reactivities.

1. Alkylating Agents

Alkylating compounds constitute an appreciable fraction of the antitumour agents known at the present time. Among the 58 preparations used and adopted for tests in the USSR and the USA and quoted in a monograph,[40] 23 exhibit an alkylating activity. In the course of metabolic reactions, such compounds form electrophilic species (carbonium ions), which are capable of alkylating DNA and enzymes, deactivating them thereby. The interest in alkylating agents as antitumour agents is associated with the hypothesis that the DNA in the vigorously dividing tumour cells is more susceptible to chemical action than in the normal cells at rest.

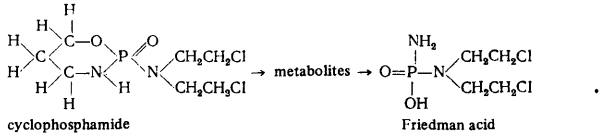
Many alkylating ATC exhibit simultaneously carcinogenic properties (the final effect of the preparation depends on the dose administered). For this reason, the conclusions and data concerning the reactivity of alkylating carcinogens are applicable to the alkylating ATC. The mechanisms of the reactions between the absolute chemical carcinogens (activated forms of carcinogens) and DNA have been examined in a review.[41] The authors note that only a small fraction of the reactions of the alkylating agents with the sites of nucleophilic attack in the DNA bases can proceed via the S_N1 mechanism. The S_N2 mechanism is commonest. Nucleic acids are alkylated to a greater extent by methylating agents than by ethylating agents. A satisfactory correlation has been observed between the carcinogenic activity of the compounds and the extent of the damage induced by them in the steric structure of the double helix of DNA.

The mechanism of the reactions of the carbonium ions CH_3^+ and $C_2H_5^+$ with guanine has been investigated by the non-empirical VRDDO method.[42] Potential surfaces for the reaction involving the addition of CH_3^+ (in the planar and pyramidal conformations) to guanine via the O[C(6)] and N(7) atoms have been calculated.



According to calculations, the attack on N(7) is preferable. The potential curves for the planar and pyramidal forms of CH_3^+ intersect at a distance of 3.7 a.u. from guanine. The intersection of the curves is due to the fact that, as it approaches guanine, the planar structure becomes less stable than the pyramidal structure. There are fundamental differences between the descriptions of the methylation and ethylation reactions of the DNA bases. The ionisation potential of CH_3^+ (IP = 9.84 eV)[42] is higher than the ionisation potentials of the DNA bases, while the opposite is true of the ionisation potential of $C_2H_5^+$ (IP = 8.38 eV). Because of this, account must be taken of the mixing of the wave functions for states with transfer of e^-, i.e. of configuration interaction, for a correct treatment of reactions of the type $A^+ + B$ in the case of CH_3^+. On the other hand, calculation for the reactions of the DNA bases with $C_2H_5^+$ can also be performed in terms of the one-determinant approximation.

A considerable proportion of alkylating agents contain the mustard gas group $N(CH_2CH_2Cl)_2$. The alkylating effect of this group is associated with the formation of a carbonium ion following the elimination of a Cl atom. Cyclophosphamide belongs to the class of widely used ATC of this kind:



The cyclophosphamide metabolites and not the cyclophosphamide itself are active in the organism. The atomic mechanism of the alkylating activity of cyclophosphamide has been investigated.[43-45] The author took into account the influence of the low-lying singlet (S_n^*) and triplet (T_n^*) excited levels on the reactivity of the ground state. The excited states play an important role in the study of dark reactions between molecules in view of their involvement in intramolecular and intermolecular charge transfer processes and spin–orbital interactions. The energies of the excited levels have been calculated by the CNDO/S method taking into account the interaction of singly excited configurations. The contribution due to the overlap of diffuse 3*d* AO of the P atom with the AO of all the atoms in the molecule was revealed by the calculations. In particular, the dipole moments of the transition between the excited levels are determined mainly (to the extent of 90%) by the interaction of 3*p* and 3*d* AO of P and 3*p* AO of Cl. The RI of the ground state (the charges and free valences on the atoms) differ little for cyclophosphamide and its metabolites—4-hydroxycyclophosphamide, 4-oxocyclophosphamide, aldophosphamide, carboxyphosphamide, and Friedman acid. The polarity (dipole moment) increases with the degree of metabolic transformations. For all the metabolites indicated, the HOMO form P = O bonds, while the LUMO are involved in intramolecular charge transfer with participation of both C–Cl groups.

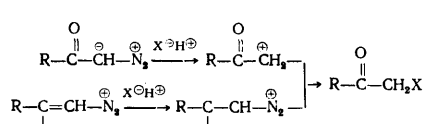
Comparison of the structures of the S_n^* and T_n^* excited levels of the cyclophosphamide metabolites and DNA bases showed that only Friedman acid has an arrangement of levels which ensures a satisfactory interaction with DNA. This conclusion agrees with data showing that Friedman acid has the most pronounced alkylating properties among all the cyclophosphamide metabolites and is at the same time capable of alkylating only nucleic acids but not enzymes. The ineffectiveness of the direct application of Friedman acid as a medicinal drug can be accounted for, according to the author,[45] by the poor transport properties of this compound in view of its high polarity.

The characteristics of the electronic structures of cytostatic agents comprising a series of phosphoric acid triamides have also been discussed.[46]

Electronic characteristics calculated by the CNDO/2 method and the antitumour activities of *p*-substituted diazoacetophenones.[4]

R	E_{HOMO}	E_{LUMO}	Atomic charge, e		Free valence index	Hammett constant σ	Inhibition of tumour growth, %	
			O	C			ARE	S-180
OCH_3	−10.959	1.916	−0.375	−0.186	0.122	−0.27	79	70
H	−11.013	1.896	−0.372	−0.172	0.114	0.00	64	63
F	−11.144	1.441	−0.343	−0.162	0.066	0.06	30	44
NO_2	−11.697	0.959	−0.324	−0.158	0.050	0.78	25	32

A scheme for the interaction of alkylating preparations in the class of diazoketones with the acid groups of biomacromolecules has been analysed by means of the CNDO/2 method:[4]



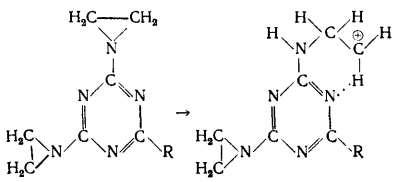
Calculation of the energies of the frontal molecular orbitals, free valence indices, and negative charges on the oxygen atom and the methylidyne carbon atom established a correlation between these parameters, the Hammett constants, and the antitumour activity of diazoketones (see Table).

Preparations containing the ethyleneimine group occupy an important place among alkylating ATC. The activation of such compounds reduces to the opening of the ring at the N–C bond in the protonated ethyleneimine and the formation of the carbonium ion $X-NH-CH_2-CH_2^+$.

Complete structural data about the disposition of all the atoms in ethyleneimine have been obtained by a non-empirical calculation in terms of the 4-21 HF basis set using gradient optimisation of the geometry.[47] The nitrogen atom is sp^3-hybridised and a value of 13.9 kcal mol^{-1} was obtained for the barrier to inversion (the experimental values are in the range 11.6–19.1 kcal mol^{-1}). The structures and thermodynamic functions of the monohydrate complexes of ethyleneimine have been calculated.[48] The energy of the electrostatic interaction for the complex with the N–H...O(I) bond, with a H_2O molecule acting as the H^+ acceptor, is −7.3 kcal mol^{-1}, while for the complex with the N...H–O(II) bond, with water as the H^+ donor, it amounts to −7.1 kcal mol^{-1}. The theoretical values of the free energies F and the equilibrium constants proved to be −0.05 kcal mol^{-1} and 1.093 for (I) and 1.42 kcal mol^{-1} and 0.092 for (II). Thus the hydration of ethyleneimine at the N–H bond is more stable.

MINDO/3 calculation for the mechanism of the opening of the ring of protonated ethyleneimine showed[49] that the direct conversion into the carbonium ion requires an appreciable activation energy (45 kcal mol^{-1}). When an external nucleophile is involved (Cl^- was used in the above study[49]), the reaction barrier is lowered to 25 kcal mol^{-1}. Thus ring opening without appreciable external influences is extremely difficult. The one-stage nucleophilic ring opening process via the S_N2 mechanism is energetically the most favourable.

Preparations of the class of substituted ethyleneiminotriazines have been synthesised:[50]



The authors postulated the formation of a stabilised intermediate form of the carbonium ion—a cyclic intermediate. The energy $E_Q = Q_C Q_N r_{CN}^{-1}$ of the electrostatic interaction of the terminal carbon atom of the carbonium ion and the nitrogen atom in the triazine ring was adopted as a characteristic of the stability of the above products. The atomic charges in the intermediate were calculated by the CNDO/2 method. Comparison of E_Q and the antitumour effect for a series of five preparations showed that the effectiveness of the preparation increases with increasing stability of the intermediate (Fig. 2). The authors suggested that this effect can be accounted for by the similarity of the structures of the cyclic intermediate and the limiting transition state in the nucleophilic attack on the molecule to be alkylated.

Certain principles of the chemical modification of the alkylating ATC have been examined.[51] Electrophiles are consumed in the organism both via non-specific reactions involving nucleophilic centres in various chemical compounds and via specific reactions with the DNA bases in tumour cells. The second channel, which is the target of chemotherapy, is much less probable than the first. An increase of the relative number of specific reactions with target molecules can be achieved, according to the authors,[51] by ensuring the preferential occurrence of the alkylation reactions via the S_N2 mechanism as a result of the intramolecular stabilisation of carbonium ions. We may note that in the general case the degree of stabilisation of such a heterocyclic intermediate increases with increase in the nucleophilicity of the heteroatom X in the sequence O < N < P < S and with decreased ring strain energy. As an example of the use of this concept, the mechanisms of the action of ATC based on phosphamide and diazoketones were examined.

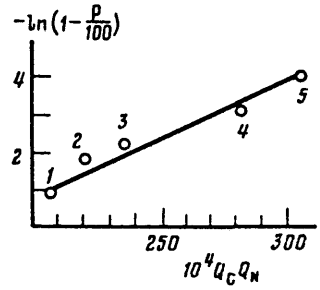
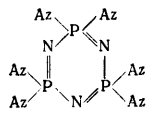


Figure 2. Dependence of the antitumour effect P of ethyleneiminotriazines on $10^4 Q_C Q_N$ (correlation coefficient 0.98): 1) C_2H_5; 2) OC_2H_5; 3) OCH_3; 4) OPh; 5) OCH_2Ph.[50]

The conformational and electronic properties of the ATC in the group of aziridinocyclophosphazans have been investigated.[52–54] Using a computer, the disposition of the ethyleneimine groups in different crystalline forms of the preparations was analysed graphically.[53] The differences between the structures of the preparations associated with the influence of the substituents and solvent molecules in the crystal lattice reduce to differences in the orientations of the aziridine rings:



Quantum-chemical calculations (CNDO/2) of the barrier of the rotation of the aziridine group demonstrated the existence of three energy minima, corresponding to crystalline modifications of the preparations. For the three preparations, $N_3P_3Az_6$, $(NPAz_2)(NSOAz)$, and $SPAz_3$ (thiophosphamide), where $Az = -NCH_2CH_2$, the atomic charges and Wiberg indices were calculated by the CNDO/2 method.[54] All the experimentally detected crystalline conformations of these ATC were examined. The reactivities were analysed from the standpoint of the possible activity of these preparations as alkylating or intercalating agents for DNA.

The alkylation of DNA by the carbonium ions derived from the ethyleneimines was examined as a process involving the coordination of Lewis acids and bases. Analysis of the atomic charges led to the conclusion that the antitumour activity is determined by the acidity of the P atom. The phosphorus atoms in the preparations $N_3P_3Az_6$ and $(NPAz_2)(NSOAz)$, which are more ative than $SPAz_3$, are correspondingly more "acid". On the other hand, comparison of the charges in other ATC—*cis*-$Pt(NH_3)_2Cl_2$ and its inactive analogue $Pt(AzH)_2Cl_2$—showed that the Pt atom has a markedly "acid" character. The total charge on the Pt atom in the first Pt salt was 0.998 (calculation by the Xα method[55]). As a result of this, the coordination of the ethyleneimine groups to the Pt atom in $Pt(AzH)_2Cl_2$ is via the unshared electron pairs of the nitrogen and the formation of the corresponding carbonium ion is difficult. According to the authors, this in fact

accounts for the low antitumour activity of $Pt(AzH)_2Cl_2$. It was concluded that the manifestation of a high antitumour activity by an ethyleneimine-containing compound requires that the atoms linked to ethyleneimine should have the optimum positive charge (−0.35e). We may note that the specific value of the optimum charge is consistent with the calculation by the CNDO/2 method.

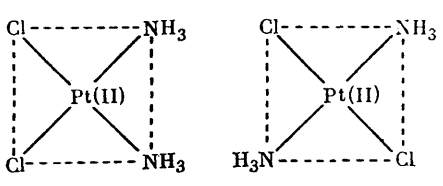
Quantum-chemical calculation (CNDO/2) of the electronic structure and stabilities of new classes of preparations of the type $[NP(CH_3)_2]_{3-x}.(NCNCH_3)_x$ and $(NPCH_3)_{3-x}.(NCNCH_3)_x$ (x = 0, 1, 2, 3) [52] made it possible to predict that the compounds with x = 1 and 2 may exhibit more effective antitumour properties than the familiar preparations $N_3P_3Az_6$. The authors' conclusions[52] have been confirmed by biological tests.

Thus the available data permit the following conclusions about the electronic mechanisms of the alkylation reactions: (1) in a specific calculation of the reaction mechanisms, it is apparently essential to take into account charge transfer states; (2) in ethyleneimine-containing ATC, the atoms close to this group play an important role. In particular, since the opening of the aziridine ring requires the involvement of an external nucleophile,[49] the nucleophilic centres of the molecule itself can in our view function as such a nucleophile. These centres are capable of subsequent participation in the intramolecular cyclisation of the carbonium ion, stabilising it, which can apparently promote the selective interaction of this carbonium ion with the nucleophilic centres of DNA.[50,51]

2. The Formation of Complexes by Antitumour Compounds

According to Emanuel's classification, preparations the mechanism of whose therapeutic action is associated with the formation of molecular complexes with biomacromolecules should be classified as complex-forming ATC. At the present time this class includes metal complexes and salts (in the first place platinum complexes and gallium nitrate) and intercalating agents. The principal target of these preparations is DNA.

The most familiar of the platinum-containing preparations is "*cis*-chlorodiaminoplatinum". The ATC based on paltinum(II) form "intercalating" intermolecular and intramolecular cross-links with DNA.[56] The cross-links are formed mainly via the 6-amino-groups of the adenine residues of the opposite chains of DNA and the N(7) atoms of the guanine residues in the G–G pairs. The electronic structure and the parameters of the reactivity of the metal salts have been calculated.[55,57–64]



cis-Pt(II) *trans*-Pt(II)

The electronic structures of the platinum complexes $PtCl_4^{2-}$, $Pt(NH_3)_4^{2+}$, *cis*-$Pt(NH_3)_2Cl_2$, *trans*-$Pt(NH_3)_2Cl_2$, and $Pt(CN)_4^{2-}$ have been calculated in terms of the approximation of the extended Hückel method (EHM) with adjustment for self-consistency on the basis of the density matrix in terms of the relativistic basis set ($5s_{1/2}$, $5p_{1/2}$, $5p_{3/2}$, $5d_{5/2}$, $6s_{1/2}$, $6p_{1/2}$, $6p_{3/2}$) and the Pt(II) AO.[57] Quasi-relativistic corrections were taken into account in terms of the "frozen" spin approximation. In constructing the Hamiltonian, corrections were introduced to take into account the external stabilising potential. The main contribution to the coefficients of the HOMO of *cis*-Pt(II) comes from the ligands, but there is an admixture of the 5*d* and 6*s* AO of platinum. On the other hand, in the case of *trans*-Pt(II) 6*p* and 5*p* AO of platinum contribute an admixture to the HOMO. The difference between the antitumour activities of these preparations (*trans*-Pt is inactive) may be associated with this factor according to the authors. Calculations were made for the complexes of *cis*-Pt(II) with guanine and the guanine-cytosine pair coordinated to the O[C(6)] and N(7) atoms. The possible mechanism of the molecular action of *cis*-Pt(II) involves the dehydrogenation of the N(1) atom of guanine, although a weakened N(1)–H bond has not been observed in the complexes investigated.

Calculations for *cis*- and *trans*-Pt(II) by the SCF-Xα method, which is widely used for calculations on organometallic compounds, did not reveal any significant differences between their electronic structures.[55] It has been suggested that the differences between the biological activities of these isomers are associated with steric and not electronic factors.

The characteristics of the structure of the hydrate complexes of dichloroaminoplatinum have been investigated[61] by the LCAO-MO method in the Mulliken–Wolfsberg–Helmholz version with adjustment for self-consistency with respect to the charges. In the complexes $[Pt(NH_3)_2XY]^{n+}$ (X, Y = Cl, H_2O; n = 0, 1, 2), the chemical bond with the ligand is formed via the 6*s* and 5*d* AO of platinum. The aquo-complexes $[Pt(NH_3)_2(H_2O)_2]^{2+}$, formed in the course of hydrolysis, possess effective electron-accepting properties and should readily interact with the nucleophilic sections of DNA. According to the calculation, *trans*-Pt(II) is more acid and is more readily hydrolysed than the *cis*-isomer. This result agrees with experimental data. It has been concluded that there is a possibility of the rapid deactivation of *trans*-Pt(II) in the organism as a result of hydrolysis, which the authors used in face to explain the absence of an antitumour effect by this compound. In our view, this explanation is better founded and more likely than the hypotheses put forward by Barber et al.[57] and Boudreaux and Carsey.[57]

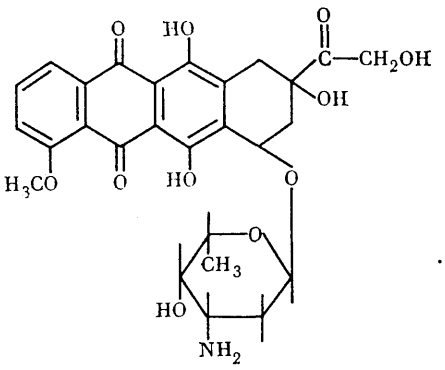
The structure–activity relations for a series of 26 dichloroaminoplatinum(II) complexes have been investigated.[62] The following RI were calculated by the INDO method (with special parametrisation for calculations on metals[63]): the frontal densities f^N, the binding energies of the complexes W_b, the molecular volume V_M, the electrostatic potential and the electric field strength E, and the energy V_2 of the polarisation interaction of the complex with a single charge. The RI were compared with the logarithms of the doses inducing the death of 50% of the animals (LD_{50}), 90% inhibition of tumours (TD_{90}), and their ratios. The two-parameter linear regression with the highest statistical reliability has the form

$$\lg(LD_{50}) = 0.0021\,(\pm 0.0008)\,W_b + 4.06\,(\pm 1.4)\,V_2 + 8.34\,(\pm 1.2),$$
$$r = 0.820,\ S = 0.37,\ F(2.23) = 23.65,$$

where r is the multiple correlation coefficient, S the standard deviation of the regression line, and $F(2.23)$ the value of the F-statistic. A relation with a correlation coefficient r = 0.817 between $\lg(LD_{50})$, on the one hand, and V_M and V_2 on the other, was also obtained. Thus the toxicity of the preparations is higher the greater the polarisability of the complex, the less stable the molecule, and the greater its molecular volume. However V_M and E_b have a high mutual correlation coefficient and the authors were unable to discover which of these quantities must be excluded from consideration. The quantity $\lg(TD_{90})$ is less well correlated with electronic parameters. The regression equation with the best correlation coefficient r = 0.626 includes E^2 and f^N.

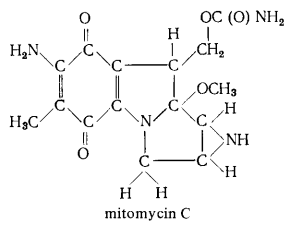
Since the electric field vector E is directed at right angles to the plane of the intercalation complex, the correlation between $\lg(TD_{90})$ and E^2 casts doubt on the intercalation mechanism of the action of *cis*-Pt(II).

The anthracycline antibiotics belong to the class of ATC capable of reacting with DNA via intercalation. The conformational analysis of anthracycline antibiotics has been carried out:[65-67]



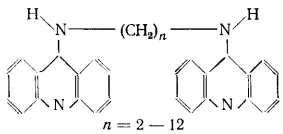
Attention has been concentrated on the determination of the equilibrium orientations of the sugar residue and the hydroxy-groups relative to the anthracycline chromophore. Calculation for adriamycin by the force field method (with and without allowance for the influence of the aqueous environment) indicates the formation of intramolecular hydrogen bonds between the hydroxy- and keto-groups of the chromophore.[66] Three equilibrium positions have been found for the carbohydrate fragment. The conformation which corresponds to that observed in the crystal has an energy higher by 3 kcal mol^{-1} than that of the lowest-energy conformation. The absolute energy minimum obtains for the conformation in which there is a hydrogen bond between the hydroxy-group in the 6-position and the O atoms of the sugar residue. The third equilbrium conformation, which corresponds to the almost parallel disposition of the carbohydrate residue relative to anthraquinone, is destabilised to the extent of 9.5 kcal mol^{-1} relative to the most stable conformation. The influence of the solvent on the relative stabilities of the conformers is insignificant. It follows from the proposed model that, for the equilibrium conformation found, there is a possibility of an additional stabilising interaction of the protonated NH_3^+ group of the sugar ring of the anthracycline and the phosphate groups of the DNA double helix. The conformations of the form of the antibiotic with a protonated sugar residue are identical to those in the neutral state.[66]

The characteristic features of the intercalation by the antitumour antibioltic mitomycin C have been investigated:[68]



The interactions of the antibiotic with the model guanidine-(3',5')-cytidine dinucleotide and all possible pairs of nucleic acid bases were examined. The intermolecular interaction energy was calculated on the basis of two additive schemes—purely empirical and imitating the results of non-empirical calculations. The orientation of the antibiotic was varied within wide limits. All the optimum positions found are located at the site of the major groove of DNA. The non-planar part of the antibiotic molecule interferes with the complete intercalation of the planar quinonoid part. The intercalation on the side of the minor groove gives rise to a relatively unstable complex as a consequence of the weak overlap of the aromatic rings. The two methods of calculation yielded somewhat contradictory data concerning the optimum orientation of the axis of the quinonoid fragment relative to the dinucleotide. An increased specificity in the intercalation of any one pair of DNA bases by the antibiotic was not found.

The relation between the antitumour activity and the characteristic features of the intercalation has been investigated for a series of diacridines.[69] The RI for the neutral forms of 27 compounds were calculated by the CNDO/3R method.



Three molecular conformations were examined: (1) the planar conformation with a symmetry axis; (2) the planar conformation with a symmetry centre; (3) the non-planar conformation with a parallel arrangement of acridine rings. These conformations correspond to different models of the intercalation by one or simultaneously two acridine rings in DNA. QSAC have been investigated for the indices of the inhibition of the *in vitro* growth of P388 cells (I_{50}) and the toxicity (LD_{10}) in mice. It follows from the analysis of the QSAC that the molecular size is correlated with $\lg(I_{50})$ and $\lg(LD_{10})$. The length of the alkyl chain between the rings does not influence the biological indices. The stability of the bonds involving the nitrogen atom in the acridine ring has a positive correlation coefficients with $\lg(LD_{10})$. For the planar forms, the energy of the N—H bond has a negative correlation coefficient with toxicity and a positive correlation coefficient with the effectiveness of the inhibition of the P388 leukaemia. According to the authors,[69] the latter factor shows that the biological activity is associated with the freedom of the rotation of the planar part of the molecule about the C—N bond relative to the intercalated acridine ring.

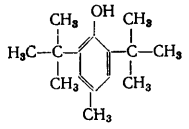
Together with the intercalation mechanism of the cytotoxic activity, alternative mechanisms of the action of a series of antibiotics are now being considered. Thus the biological effect of the anthracycline antibiotics may be based on the formation of free-radical intermediates of the semiquinone type and active forms of reduced oxygen.[70] The molecular mechanisms of the action of mitomycin C includes the formation of reactive polyfunctional intermediates—carbonium ions and semiquinone free radicals—which leads to alkylation and cross-links between the complementary chains of DNA.[71]

3. Compounds whose Activity Has a Free Radical Mechanism

It has now been established that many metabolic and pathological processes in the cell proceed via a free-radical mechanism.[1,70-72] This applies in the first place to e^- transfer reactions in the enzymic oxidation and reduction of substrates of different nature and to the lipid peroxide oxidation reactions. The products of the metabolism of many BAC are free-radical in nature (an unpaired electron is present). The intensification of the formation of free radicals in tumours is characteristic of specific stages in tumour development and this is why the inhibitors of free-radical processes can inhibit the growth of malignant formations.[1] Thus

compounds whose antitumour activity is associated with the inhibition of free-radical processes in tumour cells or is determined by free-radical products of their metabolism should be classified as ATC whose activity has a free-radical mechanism.

Compounds belonging to the group of sterically hindered phenols are included among the bioantioxidants used to fight tumour processes. The preparation dibunol (2,6-di-t-butyl-4-methylphenol) is used as an ATC in therapeutic practice.



dibunol

The conformational properties and reactivity of dibunol and its metabolites (aryloxy-radical, 2,6-di-t-butyl-4-hydroxymethylphenol, and 2,6-di-t-butyl-4-carboxyphenol) have been investigated by the MINDO/3 and INDO methods.[36] A partial optimisation of the geometry has been carried out for two conformations of dibunol, which correspond to two forms of associated species with solvent molecules. In one of them (I), the proton of the OH group lies in the plane of the benzene ring, while in the other (II) the OH group is rotated by 90° relative to the plane of the ring. According to the calculations, the conformation (II) is more stable than the conformation (I) by 1.2 kcal mol^{-1}. For both dibunol and for its aryloxy-radical, the C–O bond lies outside the plane of the ring. It follows from the MEP maps which have been constructed (Fig.3) that there is a deeper potential minimum in the conformation (II) (−95.2 kcal mol^{-1}) than in the conformation (I) (−88.4 kcal mol^{-1}). In the conformation (I) the proton and the unshared electron pair of the OH group are more shielded by the t-butyl substituents than in the conformation (II). The hydrophilic properties of dibunol and its metabolites have also been estimated.[36] The energy of the electrostatic interaction of the phenols with a water molecule averaged on the van der Waals surface was adopted as a measure of hydrophilicity. The conformation (II) is stabilised to a greater extent by the interaction with water than the conformation (I). The hydrophilicity indices are in satisfactory qualitative agreement with the experimental values of the lipophilicity parameter π for the phenols considered.

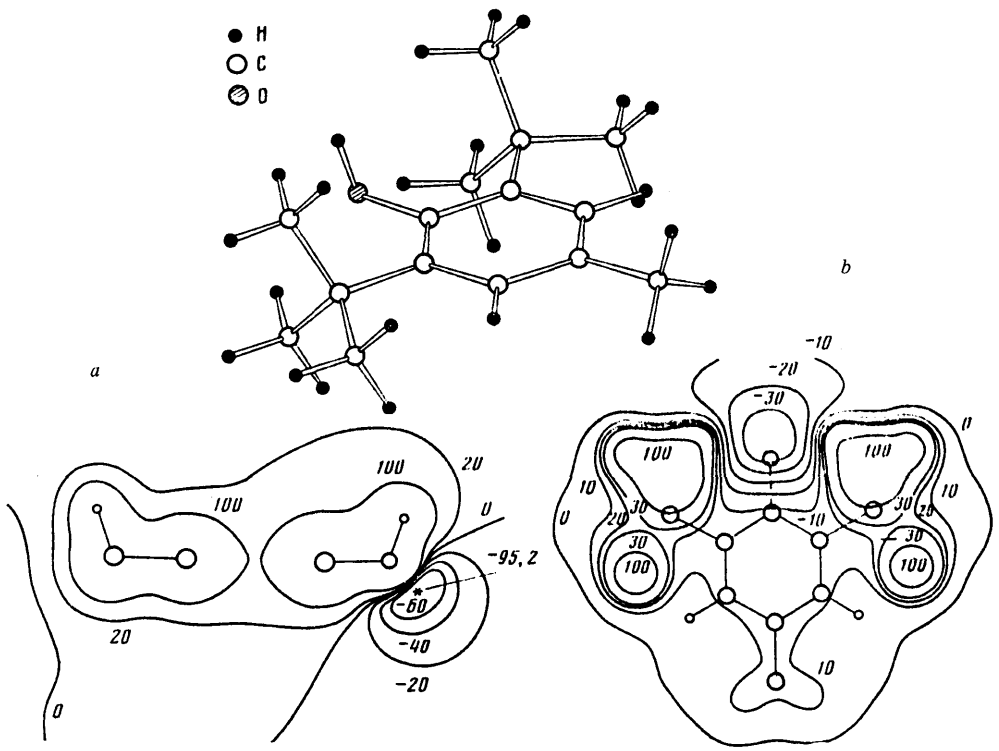
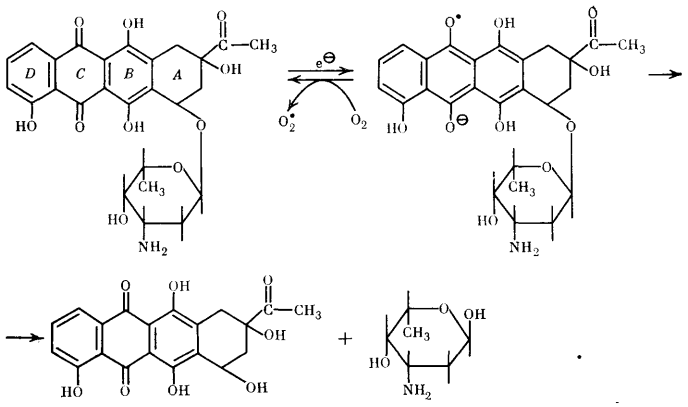


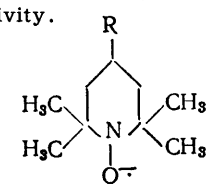
Figure 3. Projection of the three-dimensional structure of dibunol and the isopotential MEP maps (kcal mol^{-1}): *a*) in the plane of the OH group; *b*) in the plane located 1.5 Å above that of the benzene ring.[36]

The antitumour anthracycline antibiotics (AAA) may be included among the ATC whose activity has a free-radical mechanism. They act on various cell organelles—they intercalate DNA, are bound to membranes, and form radicals of the semiquinone type in enzymic reduction. The free semiquinone radicals of AAA are capable to exhibiting biological effects of two types: (1) they can generate the toxic superoxide radical-anions $O_2^{\cdot-}$ as a result of oxidation by molecular oxygen;[70,72] (2) they can bind to DNA—tentatively via an oxidative alkylation mechanism.[73]



Studies on the conformational properties of AAA and the characteristic features of the intercalation of DNA have already been considered.[65-67] The oxidation-reduction properties and reactivities of four antibiotics (rubomycin, carminomycin, adriamycin, and aclacinomycin A) and their free radicals have been investigated by the quantum-chemical INDO method and by EPR.[74,75] In order to take into account the influence of the solvent, tne hydrate complexes of the antibiotics were examined. It was shown that in the presence of H_2O molecules, the hydroxy- and keto-groups of the anthraquinone chromophore can form intermolecular hydrogen bonds with the solvent more readily than the intramolecular hydrogen bond with one another. According to the calculation, the principal splittings in the EPR spectrum of the radical-anion are associated with the protons of the *A* ring. Wiberg analysis of bond order showed that the glycosidic C–O bond between the *A* ring and the sugar residue is weakened in the radical-anions. In view of the weakening of this bond, one can postulate the possibility of the spontaneous abstraction of the sugar residue. This has been confirmed experimentally by observing the solubility of AAA in their reduction in an aqueous medium.[74] The appearance of the EPR signal of the radical-anions is accompanied by the formation of a deposit in the reaction mixture which may be associated with the elimination of the carbohydrate fragment and the formation of the antibiotic aglycones, which are sparingly soluble in water. Comparison of the energies of the frontal MO, characterising the redox properties of the AAA, with the toxic doses LD_{50} did not reveal a relation between these characteristics. Thus the differences between the toxicities of the antibiotics could not be linked by a simple relation to the capacity of their radical-anions for oxidation by molecular oxygen.

The stable nitroxy-radicals are used to modify ATC, for example anthracycline antibiotics, and themselves exhibit a weak antitumour activity.



Calculations have been made[76,77] by the INDO method of the electronic structures of 7 derivatives of the piperidin-*N*-yloxy-radicals and their reduced forms—anions and hydroxylamines. For the series of radicals investigated, a weak dependence of the electronic characteristics of the N–$O^{\cdot-}$ fragment on the nature of the substituent was observed, which has been attributed to the appreciable localisation of the MO of the oxy-group and of the substituent in the 4-position. For all the species examined, the electron in the anion is localised at the oxy-group. The redox properties of the radicals were estimated from the differences between the total energies of the anions and radicals and from the energy ε_{HOMO} of the anions and hydroxylamines. It was established that only the HOMO energies describe correctly the experimental reduction potentials. In order to estimate the transport properties of the radicals, the average electrostatic energies $W_h(R^{\cdot-})$ of the interaction with a H_2O molecule on the van der Waals surface of the nitroxy-radicals[77] were calculated analogously.[36] The electronic parameters of the compounds investigated were compared with data for their acute toxicity and antitumour activity. The inhibition coefficients κ, characterising the antitumour activity have virtually identical values (κ = 1.0–1.1) for the radicals studied. Presumably κ is determined by the electronic parameters, which are constant in this series.

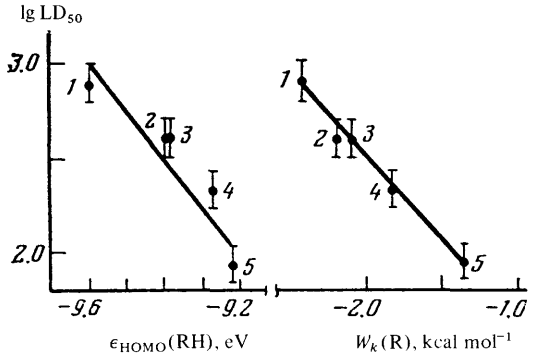
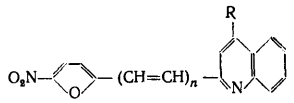


Figure 4. Dependence of the toxicity LD_{50} of piperidin-*N*-yloxy-radicals on the hydration energy $W_h(R)$ (correlation coefficient 0.99) and the energy $\varepsilon_{HOMO}(RH)$ (correlation coefficient 0.94): 1) =O; 2) OH; 3) NHC(O)$\overline{NCH_2CH_2}$; 4) OC(O)NHPh; 5) CH_2NH_2.[77]

The results of the calculations support the current hypothesis that the antitumour activity of the nitroxy-radicals is associated with their antiradical properties,[1] since the spin densities on the N–$O^{\cdot-}$ fragment, which to a large extent determine its reactivity, remains constant in the series considered. Three statistically significant relations of the type of the Hansch equation between LD_{50}, on the one hand, and $W_h(R^{\cdot-})$, $\varepsilon_{HOMO}(R^{-})$, and $\varepsilon_{HOMO}(RH)$, on the other, have been obtained.[77] According to these correlations, an increase in the hydration energy and the enhancement of the electron-accepting properties of the nitroxy-radical lower its toxicity (Fig. 4). In the organism, the radicals are rapidly reduced and exist mainly in the form of hydroxylamines. It has been concluded that the oxidation reactions of their reduced forms, leading to the formation of toxic products of the reduction of molecular oxygen, may be responsible for

the toxicity of these species. In view of the dependence of LD_{50} on $W_h(R^-)$, one can then assume that biological membranes owe the sites at which chemical reactions responsible for the toxic effect take place.

A broad spectrum of the antitumour activity (in experimental animals) is characteristic of vinylquinoline derivatives of 5-nitrofuran.

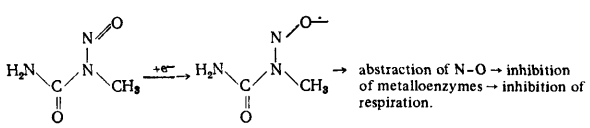


$n = 1,2$

The molecules of these compounds contain two groups which are potentially capable of exhibiting an antitumour effect—the nitro-group and the quinoline ring. It is so far not clear which of these groups is responsible for the antitumour activity. It is known that other types of biological activity of nitrofuran derivatives (antibacterial activity, the formation of nitrosyl complexes, and the generation of superoxide radicals-anions O_2^-) are associated with the reduction of the nitro-group. Quantum-chemical calculations (INDO, MINDO/3) of the electronic, conformational, and spectroscopic properties of a wide range of nitrofuran derivatives have been carried out[78] and it has been shown that the nitro-group determines the pronounced electron-accepting properties of 5-nitrofuran derivatives. The unpaired electron in the radical-anions of the vinylquinoline derivatives is localised mainly at the nitro-group (according to the calculation with allowance for the specific solvation of the NO_2 group). The electron-accepting properties of compounds of the vinylquinoline series are close to those of other derivatives of 5-nitrofuran with a high biological activity. A correlation between the electronic constants of the substituents and the antitumour activities of nitrofurans has also been observed.[79]

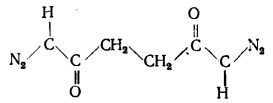
Free-radical mechanisms of the biological activity have also been proposed for two ATC derived from nitrosourea and 1,2-bisdiazoacetylethane (diazan), which have been traditionally regarded as alkylating preparations.[1,3]

The study of the electronic structures of the radical-ions of nitrosoalkylureas made it possible to predict initially[80] and then confirm experimentally the existence of a new pathway leading to the metabolic transformations of the nitrosourea, which involves the formation of nitrosyl complexes by haem-containing enzymes:[83]



The electronic parameters of the neutral state and the radical-anions of nitrosourea, in nitrosomethylurea, nitrosoethylurea, and their decomposition products (an aminoaldehyde and a nitrosamine) have been calculated by the INDO method[80,81] and by the MNDO and *ab initio* methods.[82] The electron-accepting properties and the ionisation potentials increase as follows in the series of alkyl substituents: $H < CH_3 < C_2H_5$. Both on ionisation and on capture of e^-, the greatest change in the charge takes place on the nitroso-group. The spin density in the radical-ions is also localised mainly on the nitroso-group. The mechanism of the decomposition of the radical-anions has been proposed on the basis of the analysis of the Wiberg bond orders.[80,81] When e^- is transferred to alkylnitrosourea, the N–N bond order is appreciably weakened (by 0.2 e.s.u.), i.e. elimination of the NO group becomes possible either spontaneously or via the formation of a complex with an iron atom (in model systems). In the enzymic reduction of the preparation, the formation of a complex with the Fe^{2+} atom in the active centre is apparently followed by the synchronous transfer of e^- to the preparation, a weakening of the N–N bond, and the elimination of the NO group. The formation of nitrosyl complexes leads in the first place to the inhibition of the respiratory chain, but it may be that the antitumour effect is also associated with this factor. The finding that the greatest weakening of the N–N bond is characteristic of methylnitrosourea,[80] which exhibits the greatest antitumour activity among the above three preparations, merits attention.

Quantum-chemical calculations for diazan as an ATC have been carried out.[84,85] The study of Kartsev et al.[84] was devoted to the X-ray diffraction and quantum-chemical (CNDO/2) investigation of the structure of the diazan molecule:



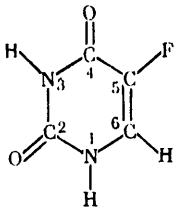
diazan

Luzhkov et al.[85] analysed the reactivity of diazan from the standpoint of its free-radical reactions. For this purpose, a study was made by the INDO and EPR methods of model oxidation-reduction reactions of these preparations. The EPR signal of free radicals of the type $X-CH_2-CH_2^{\cdot}$ (X represents the diazoketone fragment), which are capable of adding to biomolecules, was recorded in aqueous solutions of diazan in the presence of hexacyanoferrate(III), a powerful acceptor, on irradiation with light (λ = 340–380 nm). The equilibrium conformation of diazan was determined by means of a quantum-chemical calculation and the changes in its electronic structure in e^- transfer reactions were calculated. Both electron capture and electron elimination entails a rearrangement of the system of conjugated π electrons in one of the two diazoketone fragments. The maximum spin density is localised on the oxygen atom in the radical-cation. The elimination of an electron is accompanied by the destabilisation of the structure of the diazoketone fragment. According to the calculations, the diazan molecule is a weak electron donor with IP = 9.1 eV. However, when the diazo-group is displaced from the plane of the diazoketone, the ionisation potential is reduced by 0.5 eV. Presumably this conformation corresponds to the transition state in the enzymic oxidation of the molecule. Thus, as the molecular level, the activity of diazan can be attributed to its enzymic oxidation and to the alkylating activity of the $X-CH_2-CH_2^{\cdot}$ radical formed on decomposition of the diazan radical-cation.

4. Antimetabolites and Certain Other Types of Preparations

An important class of antitumour preparations comprises antimetabolites. Their activity is based on structural similarity to vitally important products of cell metabolism—nucleic acids, aminoacids, and enzyme substrates. By entering into biochemical reactions instead of the true metabolites, antimetabolites suppress the vital activity of the cell. There exist antimetabolites capable of acting selectively on actively dividing tumour cells.

Calculations have been made on 5-fluorouracil (5FU)—a preparation widely used in clinical practice.[86-89]



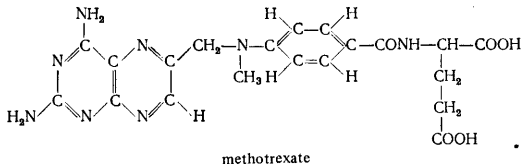
5-fluorouracil

5FU is a powerful inhibitor of the enzyme thymidylate synthetase, which is involved in an important stage in the synthesis of DNA—the methylation of uridine monophosphate to thymidine monophosphate. The differences between the reactivities of 5FU and pyrimidine bases have been deduced with the aid of quantum-chemical calculations. The relative stabilities of six lactim–lactam 5FU tautomers have been examined[89] with total optimisation of the geometry in terms of the 3-21 basis set of split Gaussian functions. The lactam form is the most stable both for the isolated tautomers and when account is taken of the interaction with water.

The reactions of uracil, thymine, 5FU, and 5-trifluoromethyluracil (which also exhibits an antitumour activity) with O_3^{2-}, adopted as a model of their interactions with thymidylate-synthetase, has been investigated.[87] As for the enzyme, a two-stage mechanism of the interaction was considered: (1) nucleophilic attack on the C(6) atom; (2) electrophilic attack on the C(5) atom in the 6-*S*-anion formed in the first stage. The quantum-chemical calculation was performed by the non-empirical method in terms of a basis set of floating spherical Gaussian functions and with subdivision of the molecule into fragments. It follows from the populations and bond orders involving the HOMO and LUMO that the specificity of the nucleophilic attack on C(6) and electrophilic attack on C(5) of the anion is determined by electronic factors. Both reactions take place with participation of π electrons. The F atom has a complex influence on the rate of the overall reaction. As a consequence of the resonance interaction, the electron density on the C(6) atom in 5FU is higher than in uracil, which should retard stage 1. On the other hand, it follows from the analysis of the electron population and the additionally calculated protonation energies that the influence of the F atom in the anion leads to an increase of the basicity of the O[C(2)] atom. The energies of the protonation of O[C(2)] decrease in the same sequence as the overall rate constants for the reactions with bisulphite: 5FU > uracil > thymine. This correlation agrees with indirect experimental data indicating the importance of the mechanism of the protonation reaction and suggests that the protonation of O[C(2)] determines the rate of the overall reaction. It has thus been explained why 5FU interacts with the enzyme more effectively than uracil. Data have also been published[86,88] concerning the interpretation of the photoelectron spectra of 5FU and pyrimidine bases as well as the energies of their complexes.

The correlations of quantum-chemical RI with the ability to inhibit dihydrofolate reductase (DHFR) have also been investigated for several classes of compounds.[90-95] DHFR is involved in the conversion of folic acid into tetrahydrofolic acid, which is essential for the synthesis of nucleic acids. Antimetabolites inhibiting DHFR inhibit the growth of tumours. In this connection new effective inhibitors of this enzyme are being sought. Calculations have been made for compounds of the class of quinazolines (series of 25 [90] and 48 [91] preparations), pteridines and triazines[97] (17 compounds), and diaminobenzylpyrimidines[93] (23 compounds). RI analogous to those for which calculations had been made by Abdul-Ahad and Webb[62] were studied in other investigations.[90-92] The potentials of interactions with trial species (H^+, O^-, CH_3^+) were calculated for diaminobenzylpyrimidines and six new descriptors were proposed on the basis of a description of the effects of interaction with the active centre. Despite the fact that statistically reliable regressions have been obtained between certain RI and the DHFR inhibition rate constants, the final conclusion concerning the mechanism of the interaction of the preparations with the enzyme was not reached. Nor is it stated whether the relations established were used to synthesise new ATC. The importance of the electronic parameters of the pyrimidine ring for quinazolines was noted. In particular, the localisation of π electrons in the pyrimidine ring is unfavourable for the manifestation of activity.[91] In the case of triazines the regressions for enzymes derived from normal and tumour tissues are very similar.[92] It was concluded that such QSAC are unsuitable for use in the search for substances with an enhanced specificity towards tumours.

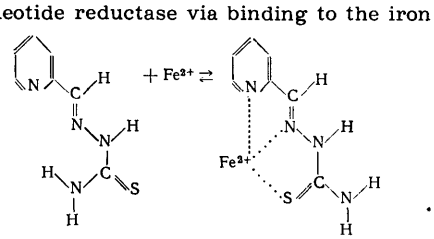
A non-empirical calculation of the energy of the binding of methotrexate, a preparation used in chemical practice, to DHFR has been carried out:[96]



methotrexate

The coordinates of the atoms of the enzyme–substrate complex were taken from X-ray diffraction data. The atoms of the active centre in DHFR were modelled by point charges, which made it possible to take into account their influence without calculating the integrals for the electron–electron interaction. It was assumed that the environment of the substrate includes 17 aminoacid residues. The methotrexate molecule was divided into six fragments because of the impossibility of carrying out the calculation for the molecule as a whole and the binding energy was calculated on the basis of an additive scheme. The value obtained (−62.69 kJ mol^{-1}) agrees with experimental estimates within the limits of 10 kJ mol^{-1}. According to the authors, the procedure proposed for the calculation of the energy of binding to the active centre is fairly simple and can be used for the screening of preparations.

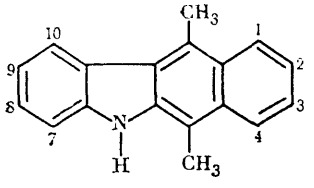
Semiempirical quantum-chemical calculations have been made[97-100] for yet another class of antitumour antimetabolites—derivatives of thiosemicarbazone (TSC). The mechanism of the action of these substances consists in the inhibition of ribonucleotide reductase via binding to the iron atom.



Calculation of the energies of the conformers of the pyridine derivative of TSC (PPP and PCILO methods with allowance for the Coulombic interaction with the solvent) showed that the given molecule has different equilibrium conformations in the free state and in the complex with Fe^{2+}.[100] The equilibrium configuration of the complex is identical with that

established crystallographically. The character of the binding of Fe^{2+} and Fe^{3+} ions to TSC derivatives has been investigated[97] by the extended Hückel method and from the electronic absorption spectra. There is a possibility of Fe^{2+} forming an ionic complex with the neutral form and a covalent complex via the S atom with the forms of TSC deprotonated at one of the nitrogen atoms. Analysis of the absorption spectra of TSC and its homologues containing the methyl or morpholinyl group instead of one of its labile protons supports the operation of a covalent mechanism of the binding of Fe^{2+} and of an ionic mechanism of the binding of Fe^{3+}. It follows from the calculations of the total energies that the Fe^{2+} complexes with neutral molecules are more stable than those with the deprotonated forms. However, the binding of iron can reduce somewhat the pK_a of the N–H group of the ligand. It follows from the EHM calculations for five pyrimidine and pyrazine derivatives that charge interactions play a decisive role for ionic binding, while the distribution of frontal densities is of decisive importance for covalent binding. Correlations between the RI of the anions of the isoquinoline derivatives of TSC (15 compounds) and their antitumour activities have been investigated.[98] On the basis of the model of the molecular activity employed, it was impossible to describe the changes in capacity to inhibit ribonucleotide reductase. Analysis of the distribution of the MO coefficients of TSC derivatives showed[99] that in the active preparations the HOMO corresponds to the unshared electron pair of the S atom, while in the inactive preparations it corresponds to the π electrons of the S atom.

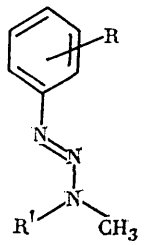
A quantum-chemical calculation (CNDO/2) for the antitumour antibiotics ellipticine, olivacine, and 2-methylellipticine has been carried out.[101] The stability of their oxidation products in the organism has been investigated. Hydroxylation proceeds most readily via the epoxide → diol (*trans*) → hydroxy-form pathway.



ellipticine

It has been shown that 9-hydroxy-isomer, which is the principal metabolite of these ATC, is less stable than the 7-, 8-, and 10-hydroxy-isomers. The antitumour activities of these compounds may be associated with the oxidation of the hydroxy-form to the quinone imine form. The ionisation potentials, calculated taking into account the solvation effects in terms of the approximation of virtual charges, did indeed show that the more active 9-hydroxyellipticine is oxidised more readily than hydroxyolivacine.

Correlations between electronic structure and antitumour activity have also been investigated for dimethyltriazene derivatives.[102–104] The molecular mechanism of the antitumour activity of these compounds is so far obscure. A series of RI have been calculated[102] for 15 derivatives of dimethylphenyltriazene.



The two-parameter QSAC obtained contain terms associated with the properties of the benzene ring, which may be due to the influence of this group on the transport of the preparation across membranes. Definite hypotheses concerning the mechanism of the biological activity could not be reached from the analysis of the terms of the regression equation referring to the triazene group Two types of QSAC have been established[104] for phenyltriazenes as a function of the type of tumour. A relation with the energy of the secondary protonation of the triazene group has been obtained for sarcoma-180, but no relation has been found between the activity in relation to leukaemia L1210 and the properties of this group. Such negative results may be accounted for by the fact that, according to Meyers,[105] phenyltriazenes may exhibit an immunostimulating activity rather than a cytotoxic activity.

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Quantum-chemical calculations in the field concerned with the synthesis of new medicinal drugs have their advantages and limitations. Modern quantum biochemistry with its great potential computational capacity can be regarded as a physicochemical approach close to the experimental one. Its objects are molecular models with a relatively small number of atoms. Quantum-chemical calculations for such models yield a general picture of the structure of electron shells and make it possible to estimate properties which are difficult to observe experimentally. Direct conclusions based on the results of calculations are concerned with the effect of BAC on biological objects at different molecular levels. It is apparently impossible to understand all the properties of the organism at the cellular and even supermolecular level on the basis of calculations for the most complex system. For example, quantum-chemical calculations are clearly inadequate for the description of the functioning of individual components of the ribosome. Quantum chemistry cannot and should not replace the experimental methods for the investigation of living organisms. Nevertheless, many events at the level of the cell and the organism ultimately reduce to processes at the molecular and electronic level. Szent-Gyorgyi stated that, whatever our approach to biology—from the side of matter or from the side of energy—we arrive at electrons in one way or another.[106] Quantum-chemical calculations are in fact most useful in the study of such phenomena.

From the investigations described above, it is evident that in recent years quantum chemists have obtained notable results in the study of the electronic structures and reactivities of the ATC and their metabolites, sections of DNA, and peptides which are vulnerable to the action of damaging factors. Interest has recently arisen in studies of the interaction of BAC with the active centres of enzymes. Such processes belong to the class which is difficult to investigate experimentally. It may be expected that quantum-chemical calculations will in fact provide in future a detailed molecular picture of enzyme processes on the basis of which it will be possible to select molecules with a potentially high specified activity, including antitumour activity.

Quantum biochemistry is developing at the junction between quantum mechanics, chemistry, and biology. For this reason, it is important to reach conclusions based on calculations for BAC in such a way that they can be understood by the majority of those who work in these fields. Problems concerning computational methods can be understood only by specialists. The results described in the language of the simplified MO theory may be accessible to a fairly large number of organic chemists and physical chemists capable of understanding the result of a theoretical study provided that it is presented in the form of the formula

of a chemical compound. Clearly, absolute conclusions about the best structure of the BAC are apparently not to be expected from specialists in the field of quantum chemistry. However, on the basis of calculations, it is possible to discover a fairly narrow range of several potentially active compounds and to employ them subsequently for experimental tests. At the present time, the majority of quantum-chemical studies concerning calculations on ATC have not as yet yielded practical conclusions. However, in many scientific centres, where there is close contact between theoreticians and experimenters, the structure–activity correlations based on RI already constitute a working instrument in the primary prediction of new active structures.

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