Molecular Orbital Analysis and Quantitative Structure-Activity Relationships for the Anthracycline Antibiotics

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Semiempirical CNDO/2 calculations of the electronic and molecular structures have been carried out for six antitumor anthracycline antibiotics. The strong correlation between their biological activity and such molecular properties as chromophore dipole moment, energy of frontier orbitals, and steric volumes was found and the proper QSAR equations were constructed. The molecular mechanics model showed the weak dependence the DNA-intercalation energy on differences in chromophore constituents (C4 and C9 positions). The expression for the intercalation energy has also been obtained on the ground of a quantum-statistical approach; it was applied to the DNA-intercalation energy calculations for the same six anthracyclines. The results agree with the molecular mechanics ones quite satisfactory.
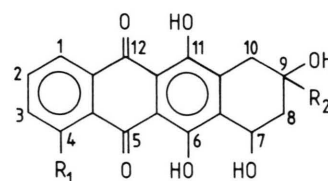
Introduction

Antitumor anthracycline antibiotics possess a striking activity and they are widely used in cancer chemotherapy. Modern molecular models of their activity are considered as involving the binding with DNA and inhibition of its matrix activity [1−3]. Small changes in molecular structure of these compounds are often followed by significant changes in their potency. At the moment more than a hundred of semisynthetical anthracyclines are accessible, however most of them have low activity/toxicity ratio, which limits their therapy. Purposeful synthesis of new effective anticancer anthracyclines with higher activity and lower toxicity turns to impossible without detailed analysis of their electronic and molecular structure (ES and MS) followed by the construction of quantitative structure-activity relationships (QSAR).

An anthracycline molecule involves two parts: chromophore anthracyclinone ring and aminosugar-daunosoamine, which are linked together with the help of an easy hydrolyzed glycosidic bond. According to experimental NMR data [4] these two parts have negligible chemical and electronical influence on each other. Crystallographic and NMR studies [1, 6] as well as molecular mechanics calculations [2, 7]

suggest that the DNA binding involves intercalation of the chromophore ring between the adjacent base pairs of DNA. According to some models an intercalation complex is characterized by an insertion of the anthraquinone ring from the major groove [1]; the other model postulates an insertion from the minor groove [5−7]. During the intercalation a chromophore ring inserts into inner hydrophobic medium of the double helix and brings forth a "nonbonded" interaction between the aromatic anthraquinone ring and base pairs. Hydrogen atom of the C9-OH group (Fig. 1) can form a hydrogen bond either with guanine of the double helix [5], or with the phosphate group of DNA [1, 5], which depends on the intercalation site. An additional bonding can also be formed between the aminosugar NH_2-group and the second phosphate group.

However, there are anthracyclines containing other sugars and retaining their antitumor activity. Following all these features of the anthracycline structure and activity we used only chromophore parameters of ES and MS for the QSAR construction and potency analysis.

Fig. 1. Chromophore of the anthracycline antibiotics.

| R_1	R_2	
1-OH	$COCH_3$	carminomycinone
2-H	$COCH_3$	4-demethoxydaunomycinone
3-OCH_3	$COCH_3$	daunomycinone
4-OCH_3	$COCH_2OH$	adriamycinone
5-OCH_3	$COCH_2OCOC_6H_5$	14-benzoateadriamycinone
6-OCH_3	$COCH_2OCOCH_2C_{10}H_8$	14-naphthaleneacetateadriamycinone

Calculations

Electronic structure calculations of the six anthra-cyclinones (chromophore part of the antibiotic) **1—6** were carried out with the help of the CNDO/2 approach [8]. ES parameters such as the dipole moment, charge distribution etc. are strongly dependent on the molecular geometry changes. X-ray data on MS obtained in [9—11] have been used for our calculations. However, all crystallographic geometries are related to the solid state. Therefore we have also analyzed the MS of anthracyclinones carrying out an additional optimization of their molecular geometry.

The chromophore is a plane molecule, except C9 atom which is shifted out of this plane and forms a "half-chair" conformation of the non-aromatic ring [9—11]. The valence structure of the chromophore aromatic part optimized in a frame of the CNDO/2 approach is in a good agreement with the experimental X-ray data [9—11].

The total energy dependence on the 9-$COCH_3$-group conformation and position of the proton in the 9-OH group (Fig. 1) was also analyzed. As postulated in [5] the proton of this group may interact with the DNA guanine during intercalation. The C9 atom which is shifted out of the molecular plane plays an ancor role and fixes the anthracycline at the described intercalation site [5]. The 9-OH group lies in the axial plane and the acetyl group — in the equatorial one. According to our calculations, this "biologically active" conformation is lower in energy as compared with the inversed one: the total energy difference is approximately equal to 2.3 kcal/mol.

An anthracycline molecule contains some intramolecular H-bonds, which influence its ES and MS. When the hydroxyl protons of the 4-OH, 9-OH and 11-OH groups were separately shifted in the perpendicular plane (as one runs out the other two groups remain their positions in the molecular plane) the chromophore total energies increase on 9.1, 8.2 and
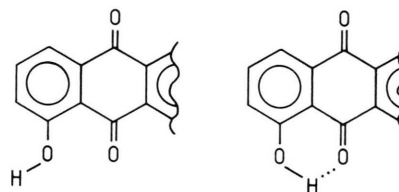
7.4 kcal/mol, respectively. Two different carminomycinone conformations are shown in Fig. 2. Both of them contain the 4-OH proton in the molecular plane. However, the right one involves an intramolecular H-bond with the C5 = O group (C—O—H valence angle in a hydroxyl group is accepted to be constant, 107°) and is more stable: the total energy difference is 6.3 kcal/mol. The intramolecular H-bond system leads to an additional stabilization of the anthracycline molecule and the plane conformation seems to be most stable and suitable for the intercalation ("biologically active").

Data on the anthraclinone MS were used for quantum-mechanical calculations of the ES of nine anthraclinones: carminomycinone, 4-demethoxydaunomycinone, daunomycinone, adriamicynone, 14-benzoateadriamycinone, 14-naphthaleneacetate-adriamycinone, 14-dihydrodaunomycinone, 5-imino-carminomycinone and 5-iminodaunomycinone. QSARs were constructed for the first six of them: **1—6**.

Although we have actually calculated the electronic structure of nine anthraclinones, only six of them have been included in a sampling set since the experimental data for these compounds satisfied the condition of being homogeneous ones: their bioactivity was established for the same tumor with the help of the same technique and by the same authors.

The QSAR analysis of the anthracycline set has been reported in [7]. The QSAR equation involves two descriptors: $\log P$, an octanol/water partitioning

coefficient, and the intercalation energy (I.E.), calculated in a frame of molecular mechanics approach. An experimental variable log*P* characterizes the relative hydrophobity, and I.E. − the DNA complexability. Strong correlation between considered QSARs parameters and antitumor potency suggests that these parameters are meaningful for the anthracycline activity explanation.

Taking into account the preceding consideration we used only chromophore parameters for a QSAR constructions, namely the calculated dipole moments μ and steric volume V_R of an radical in the 9-position of the **1−6** anthracyclines, which potency is reported in [12]. μ describes the anthracyclinone polarity and can be compared with the experimental value log*P*. The data taken from [12] represent the antitumor potency measured for leukemia cell culture in terms of ID37 (initial dose giving the cell growth inhibition by 37%). We express this variable in the standard form as $\log 1/C$ (C = ID37).

Another function of the biological activity is a retention coefficient r. It characterizes the drug quantity which being inserted in cellular inner medium is retained by the cell. Although only a small set of experimental data is given in [12], they avail a theoretical analysis. The experimental and theoretical data used for a QSAR construction are presented in Table I.

First QSAR equation for r is

$$r = (1.223 \pm 0.24) - (0.142 \pm 0.035)\,\mu + (0.008 \pm 0.001)\,V_R \tag{1}$$

Table I. Correlation of the electronic and molecular structure parameters with some data on biological activity.

	lg1/C	r	μ	V_R	ε_{int}	E_{int}
1	2.0	0.89	3.9	26.4	−30.31	−31.45
2	1.85	0.58	5.9	26.4	−29.9	−29.8
3	1.42	0.43	7.5	26.4	−31.11	−31.43
4	0.75	0.16	9.2	32.7	−31.33	−34.46
5	0.85	1.34	7.7	150.2	−31.18	−36.79
6	0.6	1.64	8.5	193.4	−31.37	−67.07

Here lg1/C is an antitumor activity, C = ID37 (10^{-6} M). r − a retention coefficient (nmol/10^6 cell), ID37 and r are taken from [12], μ − the CNDO/2 calculated dipole moment of the chromophore part (D), V_R − a steric volume calculated with the help of the van-der-Waals spheres and known interatomic distances (Å³), ε_{int} − an intercalation energy obtained in a frame of the molecular mechanics approach (kcal/mol), E_{int} − an intercalation energy on the ground of the quantum-statistical model (kcal/mol).

where V_R is a steric volume of the C9 radical. The correlation coefficient CC for this equation is equal to 0.9982. Since probability level for regression coefficients as everywhere in this paper has been taken equal to $P = 0.95$ (t-criterium), the QSAR (1) represents an adequate model. The equation (1) shows that r is growing up when V_R increases and μ decreases.

The potency (antitumor activity) function $\log 1/C$ was expressed only in terms of μ:

$$\log 1/C = (3.258 \pm 0.57) - (0.283 \pm 0.044)\,\mu$$
$$CC = 0.9125 \tag{2}$$

According to this very simple single parameter equation $\log 1/C$ like the retention coefficient r decreases at rising values of μ. We have also approximated both biological functions r and $\log 1/C$ with extended equations, which include the second power of μ. However, the accuracy increase (change in the CC value) was too small in both cases and we preferred the simpler equations (1) and (2).

The dipole moment correlates also with log*P* though with a smaller correlation coefficient $CC = 0.8$. In our studies μ has been preferred to log*P* since it is a theoretical parameter provided by quantum-chemical calculations. It also possesses a more transparent physical meaning as a polarity parameter and correlates better with $\log 1/C$ and r than log*P*.

In order to mimic the natural anthracycline-DNA staking-complex an additional molecular mechanics investigation have been carried out on the model system: anthracycline chromophore-(guanin-cytosine). The standard (1−6−12) Coulomb−Lennard-Johnes potential have been used for this purpose [13].

Both parts of the model complex (chromophore and base pair) were placed in parallel planes at the distance 3.4 Å [5] which is a distance between basepairs in the non-perturbed DNA. Other configuration parameters which define a relative orientation of two subsystems in parallel planes were obtained by the total potential energy optimization.

The minimal energy configuration was found almost identical to the experimental one [5]. The corresponding optimized geometry of the complex was used for the electronic structure calculations.

These calculations were carried out for the anthracycline chromophore which is the most simple one in the considered set: 4-demethoxy-9-deacetylcarminomycinone. The interaction energy E_{int} (which is equal to the difference: $E_{int} = E_{complex} - E_A - E_B$ and E_x is a

total energy) was calculated in a frame of three semi-empirical methods: CNDO/2, INDO and MINDO/3. It has been found, that geometry of these complex obtained with the help of molecular mechanics also corresponds to minima of the CNDO/2 and INDO potential surfaces. Actually we have analyzed only several configurations nearby the starting point (molecular mechanics configuration) and structure of the potential surface area thus obtained has allowed us to conclude that all shifts from the starting point increase the total energy. The next values for E_{int} have been obtained: -7.25 kcal/mol (CNDO/2) and -6.41 kcal/mol (INDO). The MINDO/3 approach failed to predict a complex stabilization and gave a strong repulsion between two parts of the complex: $E_{int} = 47.4$ kcal/mol for the configuration considered. The results of molecular mechanics calculations are presented in Table I; the CNDO/2 and INDO approaches gave lower stabilization energies of the intercalation complex as compared to the molecular mechanics results.

Discussion

Two most important factors which influence the anthracycline drug activity are 1) an ability to intercalate the DNA molecule as well as stability of the proper staking-complex; 2) a character of anthracycline interaction with cell membranes and/or penetration through them into the inner area of tumor cells. As a result of the penetration a drug concentration in the inner area (where the drug interacts with the DNA) increases as well as a number of intercalation modes. The problem is which of these two factors is more sensitive to anthracycline specific features. The answer on this question also provides an understanding of character of the anthracycline biological effect.

Studying the model system pointed above we have found that the anthracycline-(base-pair) complex stability is almost the same for drugs with different substituents in C4 and C9 positions (the sites of the most preferable chemical modifications). In spite of this more or less the same stability they have different antitumor activity. A strong correlation between the chromophore dipole moment and antitumor activity as well as drug retention ability demonstrates that the polarity of an anthracycline chromophore strongly influences the character of drug-membrane interaction: drug molecules with the lesser chromophore polarity accumulate preferably in the inner area of a tumor cell and therefore can produce bigger biological effect.

As far as the described staking-complex model is able to represent the real intercalation, the reported in literature attempts to increase stability of the drug-DNA complex will not produce any valuable effect. It seems that the more preferable way will be in modifying drug ability to penetrate through the cell membranes.

In conclusion one should mention, that for many biologically active compounds there were offered some QSARs like (1)−(2) which included frontier orbital energy as a descriptor. In order to interpret these relations one needs to remember the Scent-Györgyi hypothesis [14] that charge transfer has to be considered as a key process in cell functional activity as well as the Fukui frontier orbital theory [15] which explains an intermolecular interaction as an electron exchange between frontier orbitals HOMO and LUMO. Here we also try to deduce a rather simple formula which approximates the intermolecular interaction energy E_{int} on the ground of a quantum-statistical approach [16], and to apply it to estimation of E_{int} between anthracycline chromophore and base-pair in DNA. The formula includes only E_{HOMO} and orbital (spectral) densities calculated for every constituent of the complex by any semiempirical approach and looks like:

$$E_{int} = -(E^{A}_{HOMO} - E^{B}_{HOMO})^2 \cdot D_A D_B/(D_A + D_B)$$

where D_A and D_B are spectral densities.

This work is now in progress and the preliminary results are given in the last column of the Table I.

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