Modeling the Anticarcinogenic Action of Retinoids by Making Use of the OASIS Method. 3. Inhibition of the Induction of Ornithine Decarboxylase by Arotinoids†

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A series of 15 congeneric aromatic retinoids (arotinoids) was subjected to a study of the conformational dependence of basic molecular descriptors, and the anticarcinogenic potency of the compounds was modeled by the sophisticated OASIS (optimized approach based on structural indices set) method. A high correlation was obtained for both two-variable models and three-variable models. The best models of these two kinds had correlation coefficients of 0.956 vs 0.988 and standard deviations $s^2 = 0.14$ vs 0.04, respectively. The most significant variables were several interatomic and topological distances, which specify the optimum geometric drug–receptor fit. The group of significant electronic descriptors included characteristic π-bond orders, the electronic charge at one atomic position in the tetrahydronaphthalene ring, the total electronic energy, and two electronic-topological indices. An electrostatic drug–receptor interaction was conjectured on this basis. A contribution of the through-cell membrane transport was inferred from the importance of molecular refraction in the best three-variable model. The models derived were validated by the leave-one-out procedure and by reproducing the activities of five arotinoids not included in the correlation sample.

Introduction

Retinoids (vitamin A metabolites and synthetic analogs) are known as promising carcinoprevention agents, as well as drugs for treatment of several kinds of cancer and skin diseases.[1-11] Much effort is being devoted to the development of more potent and less toxic retinoids, as well as to the study of retinoid response mechanisms. Two classes of nuclear receptor proteins have been identified that are activated by retinoids: the retinoic acid receptors (RARs)[12-17] and the retinoid X receptors (RXRs).[18-22] The 3D structure of the RXR α DNA binding domain was recently determined by NMR spectroscopy,[23] and intensive studies are underway to find retinoids with high receptor selectivity.[24-26]

In the present study, as well as in the first part of this series,[27] we applied the OASIS (optimized approach based on structural indices set) method. This method was developed in 1985–86 and applied to the modeling of drug activities and toxicities of various kinds, including anticarcinogenic activity.[28-35] OASIS (like two recently developed QSAR methods[36,37]) makes use of an extended set of molecular descriptors: geometric (both topological and 3D ones), electronic (global indices characterizing the entire molecule, as well as local ones related to individual atoms and bonds), and physicochemical ones. The models thus derived yield quantitative predictions whose accuracy, in addition to fitting to experimental data, has been confirmed by comparative studies with the well-known DARC-PELCO method developed in France and successfully applied during the last 15 years in the design of new drugs.[38-40]

In the first part of our studies on retinoids,[27] we focused on a group of first-generation retinoids for which some other QSAR studies had been performed earlier.[41-43] The second part[44] dealt with the geometry optimization and conformational flexibility of more potent second-generation retinoids belonging to the class of aromatic retinoids (arotinoids).[45] Here, we present the results of an extensive OASIS modeling of a series of arotinoids, including the studying of the conformational dependence of basic molecular descriptors used in the models. A forthcoming paper will be devoted to QSAR of retinoids specifically bound to retinoic acid receptors.

Selection of Compounds and Molecular Descriptors

OASIS Descriptors. The detailed presentation of the OASIS method, and the molecular descriptors that it utilizes, was given in part 1 of this series.[27] Here we briefly comment only on the present choice of parameters. For the definition of the parameters discussed below, see references in part 1 of the series.[27]

The geometric characterization of compounds of interest was done on two levels: 2D (graph-theoretical) and 3D (spatial) ones. The selection of graph invariants or topological indices was based on previous experience and, particularly, on the significance of the descriptors in our previous study on retinoids.[27] Thus, our selection included the molecular connectivity index of Randić, χ; the valence connectivity index of Kier and Hall, χ^v; the Wiener index, W; the distance connectivity, J, and centric index $D2$ of Balaban; I'Haya's electropy, ϵ; and Bonchev's informational molecular connectivity, I_χ. Note that ϵ and χ^v are in fact combined topoelectronic indices since they associate some topological features with specific electronic distributions in the molecule.

The 3D-geometry descriptors, based on the matrix of noninteger interatomic distances, were obtained for the

† Parts 1 and 2 are refs 27 and 44, respectively.

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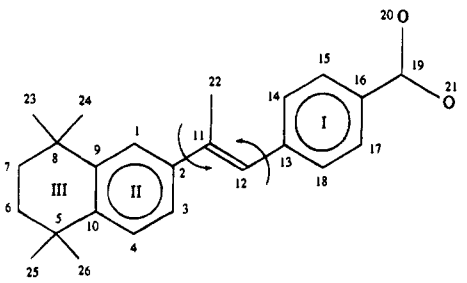

Figure 1. The atom and ring numbering in the molecular skeleton of (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB). The rotations of the two cyclic moieties are designated to occur about the 2−11 and 12−13 carbon−carbon bonds.

most stable molecular conformation by AM1 and PM3 complete geometry optimization. Included here were the largest interatomic distance, D_{max}; the 3D Wiener index, *WG*; and its information-theoretic analog, I_{WG}; as well as some interatomic distances characterizing the distance between, and the mutual orientation of, the carboxylic functionality and the hydrophobic ring *III*: $D_{C5/C19}$, $D_{C5/O21}$, $D_{C8/C19}$, and $D_{C8/O21}$ (*vide infra*). These distances were selected after a detailed examination of the sensitivity of all interatomic distances to variations in compound anticarcinogenic activity.

The quantum chemical AM1 and PM3 methods provided a detailed characterization of the electronic structure of compounds. The global electronic indices tested in our modeling were the dipole moment, μ; the total electronic energy, E_t; the calculated heat of formation, ΔH_f; the energies of the frontier molecular orbitals (HOMO and LUMO); and their gap. Atomic charges and donor/acceptor superdelocalizabilities (including the frontier orbital ones), as well as π-bond orders, were inspected as potential local electronic descriptors. Only a few of them showed any sensitivity to the variations in molecular structure occurring in the series under study: the net electronic charges on atomic positions 5 and 8; the bond order P_{5-10}; the sum of π-bond orders of bonds 1−2, 1−9, and 9−10, P_3; and the sum of all π-bond orders in aromatic ring II. (See Figure 1 for the atomic and ring numbering; in many papers the C=C bond 11−12 is referred as 9−10, following the atom numbering adopted for retinoic acid.) Consistent with our previous study on retinoids,[27] we also made use of two physicochemical properties: the hydrophobicity factor log *P* (the logarithm of the *n*-octanol/water partition coefficient *P*) and molecular refraction MRI and MRII, calculated by the atomic increments method of Ghose and Crippen.[46]

The OASIS modeling was performed by the multiple regression analysis (MRA) procedure known as the "forward/backward" or "addition/deletion" algorithm. The variables were incorporated into the models after showing the highest significance on each forward and back step, as assessed by the partial *F*-test. As in our previous study, some of the variables produced better statistics when used with a logarithmic transform.

The large number of variables (27) we selected for the arotinoid modeling is associated with the risk of producing some change correlations. In evaluating such a risk one should deal with the number of *independent* variables and not with their total number. We found more than 30 pairs of descriptors that intercorrelate with $r \geq 0.80$ (although no such pair appeared in the models reported below). Thus, for example, molecular connectivity χ intercorrelates with *W*, *WG*, I_{WG}, E_t, ϵ, and log *P*. If the intercorrelations with $r = 0.50-0.80$ are taken into account, the number of independent variables would be additionally diminished. However, even this would not have completely eliminated the risk for chance correlations. Therefore, further validation of the nonchance character of the models derived was provided by the leave-one-out procedure and by comparative predictions of the anticarcinogenic potency of arotinoids not included in the original set.

Compound Selection. QSAR modeling is based on series of congeners, i.e., compounds that have a certain similarity in their structure and elemental composition, along with some variety of structural or functional patterns. The common features are needed in order to find trends and regularities in compound activity, i.e., to arrive at a quantitative structure−activity relationship. One could hardly build a QSAR model for a series of compounds, all structural features of which are variable. One faces such a difficulty when dealing with retinoids, a class of compounds in which all three basic moieties (the hydrophobic ring, the spacer, and the polar terminus) have been subjected to a wide range of modifications. Therefore, we confined ourselves to a correlation series of aromatic retinoids (arotinoids) whose propenyl spacer and the benzoic acid moiety were kept constant but whose hydrophobic moieties varied significantly. Fifteen such compounds were found in the recent publication of Dawson et al.[47] They are congeneric with (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB), shown in Figure 1. The series thus formed is shown in Figure 2, where the numbering **1**−**15** corresponds to nos. 65, 212, 297−300, 302, 304, 214, 308, 216, 239, 312, 313, 218, respectively, of ref 47. Compound **3** is a racemic mixture of the *S*- and *R*-enantiomers **4** and **5**, respectively. Unlike other retinoids which may have many geometric isomers (diastereoisomers), arotinoids **1**−**15** can only have two such isomers.

The anticarcinogenic activities of compounds **1**−**15** were expressed in ref 47 as the inhibitory doses (ID_{50}, nmol) required to inhibit by 50% the induction of ornithine decarboxylase assay (ODC) in mouse dorsal epidermis treated with the tumor promoter TPA. In our calculations we used a negative logarithm of ID_{50}.

Conformational Flexibility of Molecular Descriptors

In part 2 of this series[44] we reported the basic results of the complete geometry optimization and conformational analysis of compounds **1**−**15**. Thus, the relative twist of the benzoic acid and tetrahydronaphthalene moieties was calculated to be 85° by the AM1 method and 56° by the PM3 method, versus the experimental value of 71° found by X-ray analysis for a solid-state configuration.[48] Sixteen conformations with very close heats of formation (mostly differing by 0−0.2 kcal/mol) were found for compounds **1**−**14**. Large rotational flexibility of the two ring fragments in each molecule (see Figure 1) was predicted by both methods. Rotational barriers of only 0.4−3.9 kcal/mol were found for the tetrahydronaphthalenyl moiety, whereas the benzoic acid moiety was found to have high barriers near 0 and 180° but to be almost free rotation in the intermediate range.

High dipole moments of all molecules studied were calculated, ranging from 3.7 to 7.6 D, which suggests a

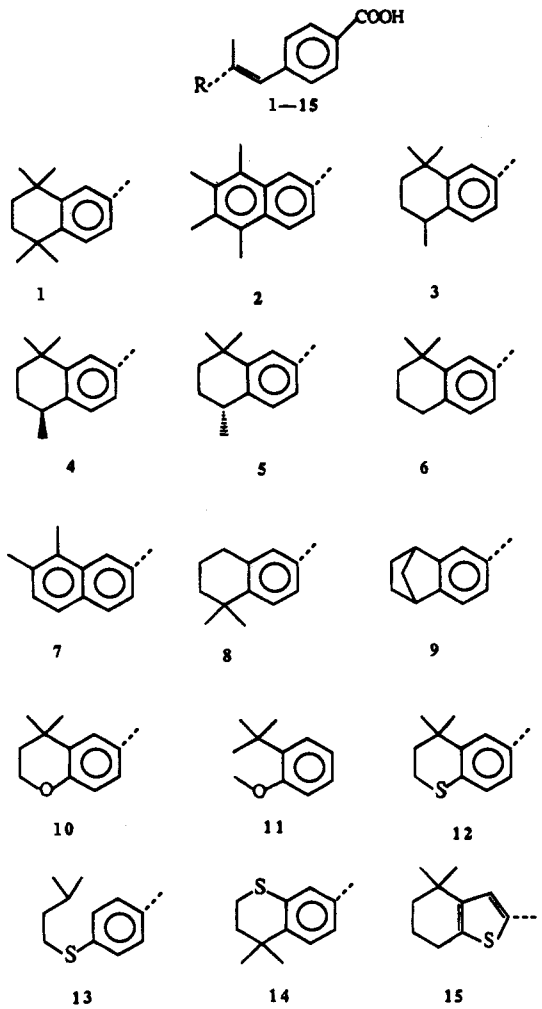

Figure 2. The 15 aromatic retinoids under study (hydrogen atoms not shown) selected from Table 18 of ref 47. The dotted lines connect the variable part of compounds 1−15 with their constant part.

nonspecific electrostatic interaction between the receptor and retinoid molecule. For most of the conformations, dipole moments determined by either method varied within a 0.4 D range. However, for compounds **12** and **13**, the conformational variations of the dipole moment were very high (2−3 D), thereby making difficult its incorporation into the models of arotinoid activity.

Electronic charges on atoms were found to change only slightly in the different conformations. As shown in Figure 3 for the 16 conformations of **1** (TTNPB), the largest differences, Δq, are less than 0.01 charge units. They refer to the carbons and hydrogens of the propenylic bridge and nearby atoms from the cyclic moieties. However, the change in the electronic charges of atoms 5 and 8, which were singled out as sensitive toward variations in molecular skeleton, was found to be $\Delta q \leq 0.002$; in other words, they may be regarded as conformationally independent. Similar insensitivity to ring rotations was observed for the bond-order descriptors P_6, P_3, and P_{5-10}.

Results and Discussion

One-Variable Correlations. The one-variable correlations are of little practical value. Rather, they present an instructive rating of the descriptors used in the subsequent multiple regression analysis. The information-theoretic analog of the Randić molecular

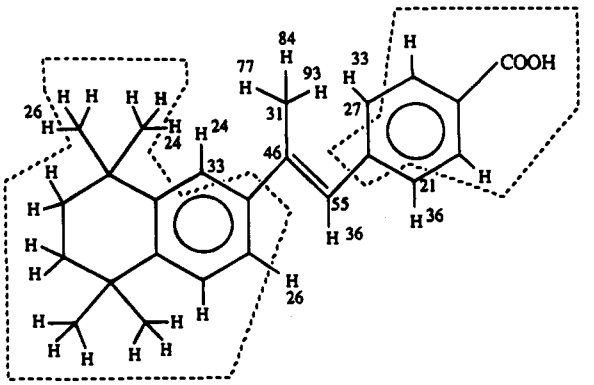

Figure 3. Largest conformational variations in the atomic electronic charges of the TTNPB molecule, $\Delta q \times 10^{-4}$ electron units. Atoms within the two dotted areas are characterized by $\Delta q \leq 0.002$.

connectivity index, I_x, is ranked first with $r = 0.80$, followed by the calculated total electronic energy, E_t, with $r = 0.74$ (PM3) and $r = 0.72$ (AM1). The maximum geometric distance (molecular diameter), D_{max}, and the energy of the lowest unoccupied molecular orbital, LUMO, come next with $r = 0.68$ and $r = 0.65$ when calculated by PM3, and $r = 0.71$ and $r = 0.77$ when AM1 is used, respectively. However, with molecule **15** included in the series, the correlation with the LUMO energies goes sharply down, thus indicating the drastic change in the electronic structure of arotinoids having a five-membered instead of a six-membered aromatic ring. The correlation with any of the three relevant interatomic distances was also worse when **15** was included, an effect caused by the smaller size of the five-membered ring. The low compatibility of the electronic and geometric structure of compound **15** with the rest of the series resulted in its inclusion into 12 but not in all 37 best models.

Two-Variable Correlations. A number of significant correlations were obtained with two variables (Table 1). The maximum interatomic distance in the moelcule, D_{max}; the total electronic energy, E_t; and the topological indices of I'Haya (ϵ), Wiener (W), Kier and Hall (χ^v), and Balaban ($D2$) were included with the highest weight in these correlations. Local electronic descriptors (charges, bond orders) and specific interatomic indices did not produce high correlation.

The pair of variables with the best statistics combines D_{max}, which simulates the arotinoid-receptor geometric fitness, and electropy ϵ, a global descriptor accounting for the σ- and π-electron distribution in the molecule. High correlation coefficients of 0.956 and 0.954 were obtained with geometries optimized by the PM3 and AM1 methods, respectively. Owing to the nonspecificity of these two parameters, correlations with only slightly worse statistics were obtained for the series including compound **15** as well (nos. 12 and 13 in Table 1). Validation of the best models for the series with 14 and 15 compounds is shown in Table 1, correlations 1 and 12, by the averaged statistics obtained by the leave-one-out procedure. The D_{max}/ϵ model is given below in its four versions:

$$-\log ID_{50} = \underset{(1.05-1.16)}{-1.13(\pm 0.13)} D_{max}^{PM3} + \underset{(33.4-42.8)}{38.9(\pm 5.8)} \log \epsilon - \underset{(74-100)}{88.8(\pm 16.9)} \quad (1)$$

Table 1. Two-Variable Correlations. The Best Model Statistics

no.	variables	correlation coefficient	standard deviation	*F*-test value	confidence interval
		$N = 14$			
1	D_{max}^{PM3}, ϵ	0.956	0.139	59	99
	(leave-one-out)	0.957	0.138	55	99
2	D_{max}^{AM1}, ϵ	0.954	0.148	55	99
3	D_{max}^{AM1}, E_t	0.925	0.236	32	96
4	D_{max}^{PM3}, E_t	0.917	0.258	29	95
5	E_t^{AM1}, W	0.918	0.256	29	94
6	E_t^{PM3}, W	0.916	0.261	29	93
7	D_{max}^{PM3}, χ^v	0.912	0.272	27	95
8	D_{max}^{AM1}, W	0.912	0.274	27	94
9	D_{max}^{AM1}, χ^v	0.911	0.276	27	94
10	D_{max}^{PM3}, W	0.895	0.324	22	92
11	D_2, ϵ	0.893	0.330	22	97
		$N = 15$			
12	D_{max}^{PM3}, ϵ	0.943	0.173	48	99
	(leave-one-out)	0.943	0.173	45	98
13	D_{max}^{AM1}, ϵ	0.941	0.177	47	98
14	D_{max}^{PM3}, χ^v	0.916	0.250	31	96
15	D_{max}^{AM1}, χ^v	0.915	0.253	31	94
16	E_t^{AM1}, W	0.906	0.279	28	97
17	E_t^{PM3}, W	0.900	0.296	26	96
18	D_2, ϵ	0.891	0.322	23	98

$$-\log ID_{50} = -1.09(\pm 0.12) D_{max}^{PM3} + 40.3(\pm 5.3) \log \epsilon - 93.3(\pm 15.3) \quad (2)$$
$$(1.03-1.12) \qquad (35.2-42.8) \qquad (79-110)$$

$$-\log ID_{50} = -1.39(\pm 0.16) D_{max}^{AM1} + 36.4(\pm 5.8) \log \epsilon - 77.7(\pm 17.0) \quad (3)$$
$$(1.29-1.42) \qquad (30.9-41.7) \qquad (63-84)$$

$$-\log ID_{50} = -1.34(\pm 0.15) D_{max}^{AM1} + 37.8(\pm 5.3) \log \epsilon - 82.3(\pm 15.7) \quad (4)$$
$$(1.26-1.38) \qquad (32.7-44.7) \qquad (69-104)$$

Equations 1 and 3 refer to the entire set of 15 arotinoids, whereas eqs 2 and 4 describe the same set without compound **15**. The coefficient ranges obtained by the leave-one-out validation procedure are shown under each equation. They are reasonably small, which justifies the use of the above equations for approximate activity assessments of congeneric yet nonsynthesized or nontested compounds. The ranges given for the first coefficient in eqs 1–4 do not include compound **13**, for which the values are 1.43, 1.32, 1.57, and 1.45, respectively. These deviations reflect the fact that the maximum distance in the most stable conformation of compound **13** is considerably longer than those of all other compounds, as shown in Table 2: compound **13** has a long isopentylthio substituent. As can be seen in Table 2, the D_{max}/ϵ models reproduce quite satisfactorily the experimetnal inhibitory doses of the arotinoids under study. Thus, for the models with 14 compounds (eqs 2 and 4) the difference between experimental and calculated activities for seven compounds is less than 0.3, and it is within the 0.3–0.6 range for the remaining seven compounds. Similarly, for the models with 15 compounds (eqs 1 and 3), this difference is less than 0.3 for eight compounds (PM3) and nine compounds (AM1); it is within the 0.3–0.6 range for five compounds (PM3) and four compounds (AM1), and for two compounds it is within the 0.6–0.7 range.

Three-Variable Correlations. The nature of the drug–receptor interaction is usually rather complex, as a result of the combined effect of different geometric and electronic factors. Therefore, it was not surprising that the OASIS model produced a number of significant three-variable correlations. Table 3 summarizes the statistics of all such models having correlation coefficients greater than 0.96 for the basic series with 14 arotinoids and greater than 0.94 for the series with all 15 compounds.

Table 2. Two-Variable Models: Experimental versus Calculated Arotinoid Inhibitory Doses (Variable Values Used in Eqs 1–4)

	inhibitory doses, $-\log ID_{50}$					variable values		
compd	expt	eq 1	eq 2	eq 3	eq 4	D_{max}^{PM3}	D_{max}^{AM1}	ϵ
1	10.52	10.77	10.75	10.72	10.71	15.05	14.91	994.5
2	8.80	8.88	8.88	8.65	8.66	16.10	15.93	954.0
3	10.15	10.13	10.08	10.16	10.11	14.85	14.73	944.8
4	10.30	9.92	9.88	9.94	9.89	15.03	14.89	944.8
5	10.22	9.90	9.85	9.95	9.91	15.05	14.88	944.8
6	8.96	8.96	8.88	9.06	8.98	15.04	14.88	893.0
7	6.70	7.29	7.22	7.31	7.24	15.88	15.66	855.6
8	9.22	8.16	8.04	8.04	7.93	15.05	14.95	893.0
9	8.51	8.95	8.88	8.96	8.89	14.78	14.88	837.1
10	8.57	9.23	9.14	9.25	9.17	14.80	14.75	893.0
11	9.40	9.56	9.47	9.52	9.44	14.61	14.63	899.0
12	9.30	9.41	9.36	9.41	9.37	15.28	15.12	931.9
13	6.77	6.54	6.60	6.63	6.69	18.05	17.29	945.9
14	9.22	9.67	9.62	9.71	9.66	15.05	14.90	931.9
15	10.00	9.30	9.21	9.33	9.25	14.72	14.67	892.1

As seen in Table 3 for the series with 14 compounds, several models were obtained. Their correlation coefficients ranged from 0.960 to 0.988, the standard deviations s^2 being within the very low range of 0.04 to 0.14, at satisfactory *F*-test values (40–133) and confidence intervals α (90–99%). When compound **15** was incorporated in the series the statistics worsened and fewer models were obtained with $r = 0.940-0.960$, $s^2 = 0.13-0.19$, $F = 29-43$, and $\alpha = 82-87\%$. This reflected the fact that the presence of a five-membered ring makes the geometric and electronic structure of this compound considerably different from those of the rest of the series.

The molecular descriptors found to be the most significant in the two-variable correlations generally maintained their importance. These were D_{max}, E_t, W, D_2, χ^v, and ϵ. However, new specific molecular descriptors were incorporated significantly into the correlations. Among these were P_6, the sum of π-bond orders

Table 3. Three-Variable Correlations: Best Model Statistics

no.	variables	correlation coefficient	standard deviation	F-test value	confidence interval
		$N = 14$			
1	D_{max}^{PM3}, MRI, P_6	0.988	0.044	133	99
	(leave-one-out)	0.988	0.043	126	98
2	D_{max}^{AM1}, E_t, $D_{C5/O21}$	0.977	0.082	69	94
	(leave-one-out)	0.977	0.081	66	93
3	D_{max}^{AM1}, W, $D_{C5/O21}$	0.975	0.087	65	95
4	W, MRII, $LUMO^{AM1}$	0.974	0.091	62	96
5	D_{max}^{PM3}, E_t, $D_{C5/C19}$	0.973	0.095	60	93
6	$D2$, W, P_6^{PM3}	0.973	0.096	59	98
7	D_{max}^{PM3}, W, P_6^{PM3}	0.972	0.099	57	96
8	D_{max}^{AM1}, E_t, $D_{C5/C19}$	0.972	0.099	57	90
9	D_{max}^{PM3}, E_t, χ^v	0.971	0.103	54	92
10	D_{max}^{PM3}, E_t, q_5	0.969	0.111	50	90
11	D_{max}^{PM3}, W, $D_{C5/C19}$	0.968	0.112	50	94
12	$D2$, E_t^{PM3}, P_6^{PM3}	0.967	0.117	48	96
13	$D2$, ϵ, P_3^{PM3}	0.964	0.125	44	93
14	$D2$, MRII, P_6^{PM3}	0.961	0.137	40	99
		$N = 15$			
15	D_{max}^{PM3}, E_t, χ^v	0.960	0.134	43	84
	(leave-one-out)	0.961	0.132	41	83
16	E_t^{PM3}, W, χ^v	0.960	0.135	43	87
17	D_{max}^{AM1}, E_t, χ^v	0.957	0.143	40	82
18	E_t^{AM1}, W, χ^v	0.955	0.150	38	84
19	D_{max}^{PM3}, E_t, q_5	0.942	0.192	29	85

of the bonds belonging to the six-membered aromatic ring II, and P_3, the respective sum of the π-bond orders of the three bonds (1–2, 1–9, and 9–10) in ring II that connect the propenylic spacer with position 5 of the hydrophobic ring III (see Figure 1 for the atom and ring numbering). The significance found for these two parameters, which may be regarded as measures of electron delocalization, parallels the experimental finding that all retinoids exhibiting anticarcinogenic effects contain a π-conjugated network of atoms, although the latter might also reflect the need for more rigid conformations. Two more electronic descriptors found a place in our three-variable correlations: the LUMO energy, which might indicate the potential importance of arotinoid electron acceptor properties, and the net electron charge of carbon atom 5, q_5.

Another group of specific molecular descriptors, which we found to be statistically significant, included two closely related interatomic distances, $D_{C5/C19}$ and $D_{C5/O21}$, i.e., two characteristic distances between carbon 5 and carbon or oxygen atoms of the carboxylic group of the benzoic acid moiety. Evidently, these distances describe in more detail the geometric fit in the first stage of the arotinoid molecule–receptor interaction.

The last parameter to be mentioned in relation to our three-variable correlations is molecular refraction, MR. As in our previous study on retinoids,[27] MR was incorporated in our best model for the series with 14 compounds (no. 1 in Table 3, and eq 5).

The best three-variable models for the series with 14 compounds are presented below by eqs 5 and 6, whereas eq 7 is the best model for the entire series of 15 compounds.

$$-\log ID_{50} = \underset{(1.06-1.10)}{-1.08(\pm 0.07) D_{max}^{PM3}} + \underset{(5.7-7.9)}{6.93(\pm 0.73) P_6^{PM3}} + \underset{(36.6-41.9)}{40.1(\pm 3.2) \log MRI} - \underset{(67-75)}{71.2(\pm 6.7)} \quad (5)$$

$$-\log ID_{50} = \underset{(22.6-28.1)}{-1.29(\pm 0.13) D_{max}^{AM1}} + \underset{(4.2-6.6)}{24.9(\pm 2.8) \log E_t^{AM1}} + \underset{(1.19-1.38)}{5.30(\pm 1.14) D_{C5/O21}^{AM1}} - \underset{(129-165)}{151(\pm 19)} \quad (6)$$

$$-\log ID_{50} = \underset{(0.96-1.10)}{-1.04(\pm 0.12) D_{max}^{PM3}} + \underset{(10.6-16.4)}{12.7(\pm 3.8) \log E_t^{PM3}} + \underset{(20.0-28.8)}{23.7(\pm 5.1) \log \chi^v} - \underset{(48-68)}{53.7(\pm 15.5)} \quad (7)$$

The leave-one-out procedure validates the use of our three-variable models for predictive purposes. This follows from both the average statistics for the leave-one-out procedure applied to the above three models (Table 3, lines 1, 2, and 15) and the small ranges of coefficient changes in the leave-one-models given in parentheses under the respective terms in eqs 5–7. The ranges given for the first coefficient in eqs 5 and 7 do not include, as was the case with our two-variable correlations, the deviation of compound **13**, whose values are 1.30 and 1.35, respectively. This does not diminish the applicability of our models because not only does compound **13** have a considerably larger D_{max} value than all other compounds in the series under study, but it is also an inactive compound. The search for highly active modifications of the known arotinoids will certainly exclude the candidates whose D_{max} values are outside a certain optimum range, found in our studies to be 14.60–15.30 Å (PM3) and, correspondingly, 14.60–15.10 Å (AM1).

Table 4 presents a comparison between the experimental arotinoid activities and those calculated by eqs 5–7, along with the values of the variables used. No calculations were made for compound **15** by models 5 and 6 because the five-membered ring of this molecule lacks the P_6 descriptor and produces a $D_{C5/O21}$ descriptor considerably smaller than those of molecules **1**–**14**. As seen from the table, the three models reproduce fairly

Table 4. Three-Variable Models: Experimental versus Calculated Arotinoid Inhibitory Doses (Variable Values Used in Eqs 5–7)

	inhibitory doses, $-\log ID_{50}$				variable values				
compd	expt	eq 5	eq 6	eq 7	MRI	P_6^{PM3}	E_t^{AM1}	$D_{C5/OH}^{AM1}$	W
1	10.52	10.72	10.82	10.78	108.8	2.417	32439	13.04	1758
2	8.80	8.61	8.55	8.78	113.1	2.179	30296	13.00	1788
3	10.15	10.25	10.23	10.19	104.8	2.413	30065	13.04	1600
4	10.30	10.08	9.98	10.01	104.8	2.417	30102	13.03	1600
5	10.22	10.04	10.04	9.98	104.8	2.415	30075	13.04	1600
6	8.96	9.22	9.13	9.08	100.0	2.413	27914	13.02	1444
7	6.70	6.94	7.15	7.16	101.3	2.180	26155	12.97	1461
8	9.22	9.23	9.13	9.05	100.0	2.415	27732	13.05	1468
9	8.51	8.38	8.15	8.47	93.7	2.412	25128	13.05	1300
10	8.57	8.41	8.71	8.93	97.0	2.335	28176	12.89	1444
11	9.40	9.29	9.28	8.96	99.2	2.375	28709	12.93	1456
12	9.30	9.24	9.63	9.53	102.6	2.387	27808	13.18	1444
13	6.77	6.74	6.70	6.54	104.2	2.417	27238	13.20	1672
14	9.22	9.50	9.15	9.73	102.6	2.389	27620	13.05	1468
15	10.00	–	–	9.45	–	–	26333	12.12	1289

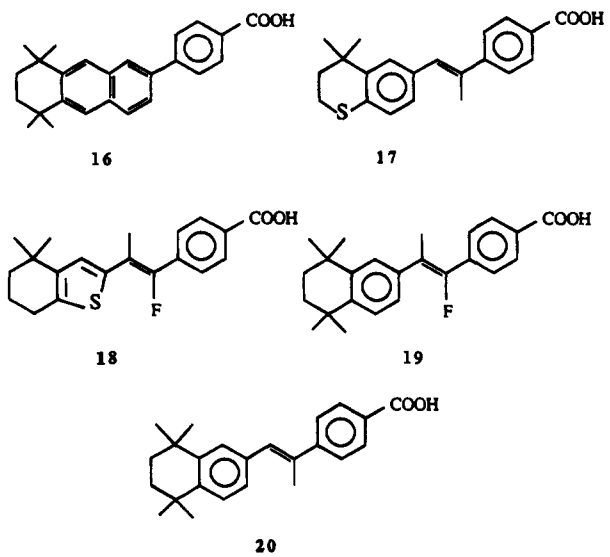

Figure 4. Arotinoids **16–20** used for testing the predictive power of the obtained OASIS models of anticarcinogenic activity.

well the experimental $-\log ID_{50}$ values, the mean difference being 0.16 for model 5, 0.21 for model 6, and 0.26 for model 7 without any outlier.

Model Testing via Structural Variations. Besides the validation made by the leave-one-out procedure for **all** models presented in Tables 1 and 3, we tested the capability of our models to reproduce $-\log ID_{50}$ values of several compounds with known high activities, not included in our arotinoid series **1–15**. These compounds, corresponding to compounds **320**, **328**, **331**, **220**, and **316** in ref 47, and denoted hereafter as compounds **16–20**, are shown in Figure 4. Differing from compounds **1–15**, in which the structural modifications occur only in the hydrophobic ring, the five test compounds have a modified spacer between the two aromatic moieties, which introduces an additional challenge for prediction purposes: compounds **18** and **19** have a fluorine substituent at the double bond (position 12, Figure 1), **17** and **20** have the methyl group attached to position 12 instead of to position 11, and **16** has a cyclized propenylic spacer that forms a tetrahydroanthracenic moiety. Like arotinoids **1–15**, compounds **17–20** can only have two diastereoisomers, whereas **16** has none.

In calculating the activities of compounds **16–20** by means of the models summarized in Tables 1 and 3, we restricted ourselves to interpolations within the ranges

Table 5. Experimental versus Averaged Anticarcinogenic Activities $-\log ID_{50}$ of Arotinoid Compounds **16–20**, Predicted by Our Two-Variable and Three-Variable Models

compd	expt	two-variable models	three-variable models	average	difference
16	10.15	11.08 (2)[a]	10.54 (8)	10.65 (10)	+0.50
17	9.22	9.34 (18)	9.58 (15)	9.45 (33)	+0.23
18	9.22	10.28 (16)	9.45 (7)	10.03 (23)	+0.81
19	10.10	10.35 (4)	12.00 (4)	11.18 (8)	+1.08
20	10.52	10.53 (18)	10.75 (16)	10.63 (34)	+0.11

[a] Numbers in parentheses stand for the number of models obeying the condition for interpolation of parameters.

of the variable values specified for compounds **1–14** and **1–15**, respectively. When one or more variables did not obey the above condition (such cases were treated as "extrapolations"), the models were discarded in order to diminish the risk of unreliable predictions. This reduced the number of models used in the calculations to eight for compound **19** and to 10 for compound **16**, whereas for compounds **18**, **17**, and **20** this number remained sufficiently large (23, 33, and 34, respectively). The results are summarized in Table 5, in which the values of the calculated activities were averaged over the two-variable and three-variable models, as well as over all models used.

As seen in Table 5, the averaged predictions made by both the two-variable models and the three-variable ones agree very well with the experimental values for compounds **17** and **20**. For the other three compounds the two groups of models differ significantly in their predictions. The two-variable models produced a fairly accurate activity value for compound **19**, but the respective activities of **16** and **18** were overestimated by 0.93 and 1.06, respectively. Curiously enough, the activities of these three compounds were calculated by the three-variable models in a reversed order: quite acceptable values for compounds **16** and **18** but a highly overestimated one for compound **19** which has the fewest models. As a result, the calculated total average activity of compound **16** may still be regarded as satisfactory (10.65 vs the experimental value 10.15). The difference between the experimental and calculated activities of the two fluoro-substituted compounds **18** and **19** is, however, rather large (0.81 and 1.08, respectively).

That the basic series does not contain any fluorine (or other halogen) atom cannot explain completely the above-mentioned discrepancy. (Unlike Hansch's method, OASIS does not deal with substituent contributions but describes the molecule as a whole. Therefore, there is no specific requirement that tested substituents be

included in the series.) One may also take into account the difficulties in reproducing the activity of compounds containing a five-membered ring instead of a six-membered one, a problem already faced when dealing with compound **15** (**15** and **18** are the only such compounds). However, it seems important to try to understand why all averaged predicted activities are higher than the experimental ones. The inspection of the different types of models revealed that models that incorporate interatomic distances generally overestimate the calculated activity. The distance descriptors (D_{max}, $D_{C5/C19}$, and $D_{C5/O21}$, as well as the indices W and D_2, based on topological distances) are included in most models, usually with the highest weight, thus determining the general trend, despite the opposite trend (with lower weight) displayed by bond-order descriptors. Being aware of the tendencies of different types of descriptors to overestimate or underestimate activity value, one may be able to introduce corrections in the right direction.

On the other hand, each model has its limited predictivity area. The limitations of our model stem from the series selected, in which structural modifications were allowed only for the tetrahydronaphthalene moiety (preserving, however, its aromatic ring) but not for the propenylic spacer or benzoic acid fragment.

Additional calculations performed for three other compounds suggested by a reviewer showed low agreement with the measured activities because they involve more pronounced spacer modifications (namely the substitution of the tetrahydronaphthalene ring by a geranylidene ring, the substitution of the benzoic acid moiety by a naphthalene one, or the addition of a second methyl group to the spacer (compounds **64**, **242**, and **318** from ref 47)).

The predictivity of our models was thus specified mainly within structural modifications of the tetrahydronaphthalene ring and less essential changes in the propenylic spacer. The next question of importance was to what extent the models can help in the search for enhanced bioactivity. As illustrated by eq 1–7, our two-variable and three-variable models include both positive and negative terms. Hence, any modification that increases E_{tot}, MR, ϵ, χ^v, P_6, q_5, and $D_{C5/COOH}$, and simultaneously decreases D_{max}, W, $D2$, and LUMO, could potentially result in a more active arotinoid. The fact that any decrease in overall molecular size (D_{max}, W, $D2$) must be associated with an increase in the distance between carbon atom 5 and the carboxylic group indicates the presence of a narrow optimal range of interatomic distances for which both conditions can be met. Similarly, the requirement for an increase in MR or E_{tot}, which means more atoms or electrons, in combination with a decreased molecular diameter imposes another optimum range of torsion angles between the two cyclic moieties and favors the incorporation of heteroatoms such as S and Cl. The latter conclusion was also supported by the requirement for an increase in ϵ and χ^v. Finally, any structural modifications that increase the electron delocalization in the aromatic part of the tetrahydronaphthalenic moiety and improve at the same time molecular electron-acceptor properties could also bring an enhanced potency.

Some Conclusions Concerning The Arotinoid-Receptor Interaction

Several types of molecular parameters were found to be significant for the anticarcinogenic activity of the arotinoids under study. A fairly complete drug–receptor geometric fit was achieved by using the topological indices of Wiener and Balaban, the maximum distance, and the distance between carbon atom 5 in the hydrophobic ring III and carboxylic functionality.

The electronic factor was also of importance. Three *global* electronic descriptors were most frequently found to be significant: the total electronic energy, the electropy, and the Kier and Hall valence connectivity. The *local* electronic factors singled out were mainly bond orders; the most significant factor of this kind was the sum of the π-bond orders in the aromatic ring from the tetrahydronaphthalene moiety. Atomic position *5*, also mentioned above as related to the geometric fit, appeared again in several models as the net atomic electronic charge q_5. These findings underscore the importance of atom 5 in the metabolic process, as mentioned by Dawson et al.[47]

On the other hand, the excess of positive electronic charge on the carboxylic hydrogen ($\approx$0.20–0.23) makes possible the hydrogen bonding to the receptor at this position. In addition, the incorporation of the LUMO energy into one of the three-variable models, as well as its appearance among the best single-variable correlations, might suggest some electron-acceptor interaction. This could be related to the importance of electron delocalization in all potent anticarcinogenic agents of this group, reflected by the above-mentioned bond-order descriptors. The AM1 and PM3 superdelocalizability indices detected no evidence for donor–acceptor interaction at this position, a possible explanation being the great difference in the values of these indices for carbon, sulfur (compounds **12** and **13**), and oxygen (compound **10**).

Hence, although not excluding the possibility of a donor–acceptor interaction and assuming the possibility of arotinoid-receptor hydrogen bonding with the carboxylic hydrogen, we may infer that the electronic interaction is mainly electrostatic, as concluded in our previous study on several first-generation retinoids.[27]

The last factor found to be highly significant in our OASIS modeling was molecular refraction, MR. Bearing in mind the close connection between the MR and the hydrophobicity factor log P, one may suppose that the correlations obtained with MR indicate the importance of the through-cell membrane transport. We could not reproduce the direct correlations with log P found in part 1 for another series of retinoids;[27] however, the methods of calculating log P are still far from perfect, and more definite conclusions of this type should be based on experimental log P values.

Summarizing the above discussion, one may specify that the arotinoid (retinoid) interaction with the bioreceptor includes (i) a geometric fit determined by the distances between carbon atom 5 in the hydrophobic ring and the carboxylic moiety, (ii) a predominantly electrostatic interaction involving an active role for atom 5, (iii) a possible role for the through-cell membrane transport.

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