

Discrimination and Molecular Design of New Theoretical Hypolipaeic Agents Using the Molecular Connectivity Functions

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The molecular topology model and discriminant analysis have been applied to the prediction and QSAR interpretation of some pharmacological properties of hypolipaeic drugs using multivariable regression equations with their statistical parameters. Regression analysis showed that the molecular topology model predicts these properties. The corresponding stability (cross-validation) studies done on the selected prediction models confirmed the goodness of the fits. The method used for hypolipaeic activity selection was a linear discriminant analysis (LDA). We make use of the pharmacological distribution diagrams (PDDs) as a visualizing technique for the identification and design of new hypolipaeic agents.

The QSAR (quantitative structure–activity relationship) methods examine the relationship between a determined property and the chemical structure of a series of molecules,¹ thus making it possible to observe the variation in this property in the analyzed series. This information can then be used to predict the values of this property in other molecules of the same therapeutic group.

Molecular connectivity is a topological method able to describe the structure of a molecule by means of numbers called indices (${}^m\chi_i$), calculated from the hydrogen-suppressed graphs of the molecule being studied. These indices subsequently regress in relation to the experimental values of the physical, chemical,² and/or biological^{3,4} properties to obtain the so-called connectivity functions.⁵

We chose a group of hypolipaeic drugs (drugs that decrease the lipid concentration in plasma) to study QSAR relationships and the corresponding connectivity functions, demonstrate the predictive capacities of both, and obtain a discriminant function that facilitates the design and molecular selection of new structures with theoretical hypolipaeic activity.⁶

MATERIALS AND METHODS

In the connectivity method, molecular structure is expressed topologically by the hydrogen-suppressed graph. It should be noted that information concerning the contributions of the hydrogen atoms is implicit in this graphical formulation. For alkanes there is a direct relation between the vertex valence δ_i and the number of hydrogen atoms (H) implied at vertex i , $\delta_i = 4 - H$. The number 4 may represent the valence or the number of valence electrons for the carbon atom.

The ${}^m\chi_i$ are terms defined by a subgraph of type t containing m edges, connected in the graph. Disconnected subgraphs are not considered. The order of the subgraph is

	Order (m)				
Type (t)	0	1	2	3	4
Path (p)					
Cluster (c)					
Path-cluster (pc)					
Connectivity indices	${}^0\chi = 4.284$	${}^1\chi = 2.270$	${}^2\chi = 1.802$	${}^3\chi_p = 0.816$ ${}^3\chi_c = 0.408$	${}^4\chi_p = 0.000$ ${}^4\chi_c = 0.000$ ${}^4\chi_{pc} = 0.408$

Figure 1. Subgraphs and connectivity indices of isopentane.

defined as m . Subgraphs may be classified into four types: (1) path (p), subgraphs whose subgraph valences are no greater than 2; (2) cluster (c), subgraphs whose subgraph valences include at least one of 3 or 4 but do not include 2; (3) path-cluster (pc), subgraphs whose subgraph valences must include 2 in addition to 3 and/or 4; (4) chain (ch), edge sequences containing at least one cycle.

The connectivity indices (${}^m\chi_i$) proposed by Kier and Hall⁷ and based on the Randic index⁸ are evaluated as a sum of terms over all the distinct connected subgraphs and are defined by the general equation

$${}^m\chi_i = \sum_{j=1}^{n_m} {}^mS_j \quad (1)$$

where m = order of a subgraph, i.e., number of edges of a subgraph, n_m = number of type t subgraphs of order m , and

Table 1. Experimental Values of Several Pharmacological Properties of a Group of Hypolipaeic Drugs Used in the Connectivity Functions

compd	EGC (%)	RTC (%)	PPB (%)	T_{\max} (h)	LD-50m (g/kg)
acifran			88.0	1.50	
benfluorex		14.00		1.50	
bezafibrate	22.00	18.00	95.0	1.75	
ciprofibrate	73.00	21.00	69.5	2.00	
clonofibrate		11.00		5.00	
clofibrate	83.00	15.00	96.4	4.00	2.46
doxazosin		8.00	98.5	3.00	
fenofibrate		20.00	99.0	5.00	5.00
gemfibrozil	50.00		96.0	1.50	3.16
lovastatin		27.00	95.0	4.00	
niceritol		16.00			
nicotinic acid		10.00			
pantethine		21.00			10.00
pirifibrate					1.25
plafibrade					3.78
pravastatin		16.00	55.0	1.25	9.76
probucol		24.00	5.0	24.00	5.00
simfibrate				6.00	3.40
simvastatin		25.00	95.0	1.85	
tiadenol	10.00	25.10			

mS_j = quantity calculated for each subgraph and defined by

$${}^mS_j = \left[\prod_{i=1}^{m+1} (\delta_i) \right]^{-1/2} \quad (2)$$

where j denotes the particular set of edges that constitute the subgraph.

All the distinct subgraphs and connectivity indices for isopentane are shown in Figure 1.

The vertex valences, δ^v , of the unsaturated carbon atoms and the heteroatoms (N, S, O) can be calculated using

$$\delta^v = Z^v - H \quad (3)$$

where Z^v is the number of valence electrons of the atom and H is the number of hydrogen atoms attached to it.^{4,8} The empirically derived values for the halogens were also used.⁵

We used 16 ${}^m\chi_i$ valence and nonvalence indices in our study, ranging from zero to fourth order, whose types are path, cluster, and path-cluster.

A multiple regression analysis was used to find the relationship between the pharmacological properties of hypolipaeic drugs and the connectivity indices. It can be expressed as⁷

$$C(\chi) = P = A_0 + \sum_{i=1}^n A_i \cdot \chi_i \quad (4)$$

where P is a property and A_0 and A_i represent the regression coefficients of the obtained equation. Once the connectivity function (eq 4) is established, its value for a specific molecule may be predicted.

The connectivity indices that were used in this study were calculated using eqs 1–3 and computer software developed in our department.⁹ The connectivity function (eq 4) was obtained by multilinear regression with the BMDP 9R program of the biostatistics package BMDP (Biomedical Computer Programs).¹⁰ To test the quality of the regression equations, the following statistical parameters were used: multiple correlation coefficient (r), standard deviation (sd), F -Snedecor function values (F), Mallow's CP, and Student's t -test (statistical significance), as well as the corresponding cross validation of the selected functions.

Cross-validation for the selected functions was carried out using the jackknife method.¹¹ With this method, n observations are eliminated by means of a random process, and a regression program is applied. The process was repeated as many times as necessary until all the observations were eliminated at least once and at most four times. Finally, the coefficients of the independent variables calculated, the correlation coefficients, standard deviations, and the residuals are compared with those obtained in the selected equation.

The following pharmacological properties were investigated: (1) elimination as conjugated glucuronide (%), (2) reduction in total cholesterol (%), (3) percentage of plasmatic protein binding (PPB), (4) maximum plasmatic concentration time (T_{\max}) (h), (5) lethal dose 50 p.o. in mouse (LD-50m) ($\text{g} \cdot \text{kg}^{-1}$). The experimental values for these properties were obtained from different bibliographic sources^{12–20} (Table 1).

Linear discriminant analysis (LDA) was used to select the parameters that identify the active or inactive character of the molecules, with the help of the BMDP 7M program,

Table 2. Connectivity Index Values of a Group of Hypolipaeic Agents Used in the Correlation Equations

compd	${}^0\chi$	${}^0\chi^v$	${}^1\chi^v$	${}^2\chi$	${}^2\chi^v$	${}^3\chi^v$	${}^3\chi^c$	${}^4\chi^v$
acifran	10.085	8.636	4.778	4.850	3.679	2.582	1.031	0.005
benfluorex	16.516	13.845	7.977	8.475	5.759	3.634	1.948	0.013
bezafibrate	16.361	14.909	8.242	7.980	6.551	3.892	1.744	0.102
ciprofibrate	12.508	11.765	6.450	7.328	6.718	2.518	2.500	0.102
clonofibrate	22.897	20.596	12.143	11.779	9.675	7.461	2.640	0.207
clofibrate	11.138	10.445	5.485	5.400	4.263	2.229	1.367	0.102
doxazosin	20.922	18.484	10.686	10.719	7.810	5.877	1.355	0.000
fenofibrate	16.524	15.533	8.406	8.304	6.904	3.706	2.030	0.102
gemfibrozil	12.767	11.617	6.262	6.301	5.410	3.126	1.547	0.177
lovastatin	19.922	18.303	11.479	11.162	9.636	7.320	1.941	0.000
niceritol	24.532	21.621	12.243	11.378	8.771	5.627	1.540	0.125
nicotinic acid	5.594	4.612	2.438	2.146	1.547	0.908	0.260	0.000
pantethine	26.297	22.658	13.38	13.272	11.314	6.757	3.032	0.408
pirifibrate	15.154	13.779	7.493	7.323	5.898	3.331	1.602	0.102
plafibrade	16.165	14.629	8.119	8.619	6.392	3.803	1.747	0.102
pravastatin	21.215	18.107	10.747	10.675	8.460	5.932	1.932	0.000
probucol	26.224	25.714	13.767	16.150	16.941	7.834	6.981	1.505
simfibrate	20.983	19.597	10.557	10.860	8.712	4.662	2.733	0.204
simvastatin	20.844	19.096	11.353	11.307	9.831	6.846	2.508	0.177
tiadenol	13.314	12.804	8.975	5.950	6.291	4.331	0.000	0.000

Table 3. Connectivity Function Relationship Obtained by Multilinear Regression for Several Pharmacological Properties^a

property	equation	<i>n</i>	<i>r</i>	sd	<i>F</i>	CP	<i>p</i>
elimination as glucuronide conjugated (%)	EGC = $-(35.36 \pm 0.89)^3\chi^v + (161.45 \pm 2.95)$	5	0.999	1.58	1581	1.17	<0.001
reduction in total cholesterol (%)	RTC = $(0.27 \pm 0.16)^0\chi^v - (28.20 \pm 6.80)^2\chi^v + (48.11 \pm 9.26)$	15	0.829	3.56	13.16	4.00	<0.001
protein plasmatic binding (%)	PPB = $-(26.75 \pm 4.48)^0\chi + (32.00 \pm 5.27)^0\chi^v - (32.45 \pm 3.16)^3\chi_c + (111.10 \pm 7.47)$	11	0.982	6.48	63.57	4.00	<0.001
maximum plasmatic concentration time (h)	$T_{\max} = (14.67 \pm 1.16)^4\chi_c^v + (1.58 \pm 0.48)$	14	0.964	1.61	159.6	2.63	<0.001
lethal dose 50 p.o. in mouse (g/kg)	LD-50m = $-(2.57 \pm 0.58)^0\chi^v + (5.07 \pm 0.99)^1\chi^v + (0.80 \pm 1.58)$	9	0.935	1.26	20.73	2.49	<0.010

^a *n* = number of values, *r* = coefficient correlation, sd = standard deviation, *F* = Snedecor function, CP = Mallow's CP, *p* = significance.

which also belongs to the BMDP computer package. The analysis was carried out on two groups of molecules, one with demonstrated hypolipaeic activity and the other inactive. The quality of the final LDA equation obtained may be assessed in three ways: comparison of the tabulated *F* and *U* statistical values, determination of the percentage of molecules correctly classified, and prediction of the classification of molecules not included in the original study (cross-validation).

Once we had obtained the ideal discrimination conditions for classifying the hypolipaeic activity of a particular compound, the next step was to find new active compounds. For this purpose we used a molecular design software package developed in our research unit, the purpose of which is to build chemical structures starting from a base structure, to which molecular fragments are added in the bonding positions that have previously been assigned.^{6,21}

The topological indices and pharmacological parameters of computer-designed molecules were determined (with the help of the relevant connectivity functions) and were classified as active or inactive by applying to them the discriminant function.

RESULTS AND DISCUSSION

The molecular connectivity indices for the 20 hypolipaeic drugs examined in the present study are shown in Table 2.

The connectivity functions obtained by multilinear regression and the statistical parameters for several pharmacological properties are shown in Table 3.

Cross-validation for the equations was carried out by varying the number of eliminations and the number of runs for each specific property. We found that raising the number of eliminations made the model more unstable, which, on the other hand, was to be expected because the degrees of freedom were considerably diminished. Of the five equations, all of which were completely stable, we give the cross-validation only of the three latter selected by LDA. Thus, in the case of the percentage of plasmatic protein binding and maximum plasmatic concentration time the stability corresponding to two eliminations was chosen, and the process was repeated a total of 11 and 14 runs, respectively. In the case of lethal dose 50 p.o. in mouse the stability corresponding to one elimination was chosen and the process was repeated a total of nine runs. This means that, in all cases, approximately 10% of the observations were eliminated, and this value coincides with the one recommended by some authors⁴ (Tables 4–6). Comparison between the values

Table 4. Cross-Validation (Jackknife Method) for the Regression Model Corresponding to the Plasma Protein Binding (PPB) Values of Hypolipaeic Drugs

	original model (no deletions)		2 deletions per run (11 runs)	
	regression value	sd	regression value	sd
correlation coefficient	0.982		0.981	0.015
sd	6.485		6.700	0.829
coefficient of $^0\chi$	-26.754	4.481	-27.052	3.663
coefficient of $^0\chi^v$	31.998	5.271	32.417	4.283
coefficient of $^3\chi_c$	-32.450	3.165	-32.302	2.298
constant	111.091	7.471	109.628	6.195
average residual	3.999	1.038	4.533	0.881
residuals < 1 sd (%)	81.82		75.21	
residuals between 1 and 2 sd (%)	18.18		22.31	
residuals > 2 sd (%)	0.00		2.48	

Table 5. Cross-Validation (Jackknife Method) for the Regression Model Corresponding to the T_{\max} Values of Hypolipaeic Drugs

	original model (no deletions)		2 deletions per run (14 runs)	
	regression value	sd	regression value	sd
correlation coefficient	0.964		0.918	0.186
sd	1.609		1.651	0.088
coefficient of $^4\chi_c^v$	14.670	1.161	14.017	2.356
constant	1.580	0.486	1.685	0.222
average residual	1.262	0.219	1.332	0.252
residuals < 1 sd (%)	71.43		70.41	
residuals between 1 and 2 sd (%)	28.57		29.08	
residuals > 2 sd (%)	0.00		0.51	

Table 6. Cross-Validation (Jackknife Method) for the Regression Model Corresponding to the LD-50m Values of Hypolipaeic Drugs

	original model (no deletions)		1 deletion per run (9 runs)	
	regression value	sd	regression value	sd
correlation coefficient	0.935		0.934	0.020
sd	1.261		1.267	0.153
coefficient of $^0\chi^v$	-2.570	0.577	-2.607	0.235
coefficient of $^1\chi^v$	5.076	0.993	5.135	0.398
constant	0.800	1.580	0.857	0.622
average residual	0.868	0.196	0.880	0.033
residuals < 1 sd (%)	77.78		76.54	
residuals between 1 and 2 sd (%)	22.22		22.22	
residuals > 2 sd	0.00		1.23	

obtained with the selected model and the model of one or two eliminations, depending on the specific case, shows that the selected equations are very stable. This is made patent by the equality of the obtained terms, as well as by the low standard deviations in each of them. Analysis of the obtained residuals with the selected model as well as for the one- or two-elimination model reveals minimum discrepancies in the

Table 7. Selected Parameters in the Final Equation Obtained by Linear Discriminant Analysis (LDA) Applied to Hypolipaeic Drugs^a

parameter description	coefficient	F to remove	U statistical
protein plasmatic binding (PPB) (%)	0.001 87	0.1048	0.9141
maximum plasmatic concn time (T_{\max}) (h)	0.191 83	4.5419	0.9307
lethal dose p.o. mouse (LD-50m) (g/kg)	0.054 05	0.9826	0.9157
constant	-0.981 46		

^a Discriminant function: $\Delta P = 0.00187\text{PPB} + 0.19183T_{\max} + 0.05405\text{LD-50m} - 0.98146$.

Table 8. Results Obtained by Linear Discriminant Analysis, Carried out with 29 Different Compounds with Hypolipaeic Activity and 34 Different Inactive Compounds

compd	active group classif	ΔP	probability (%)	compd	inactive group classif	ΔP	probability (%)
clofibrate	—	-0.10	48	flufenamic acid	—	-0.40	40
bezafibrate	U	0.02	50	acetylsalicylic acid	—	-0.45	39
plafibride	U	0.01	50	aminopyrine	—	-0.37	41
pirifibrate	—	-0.03	49	diclofenac	—	-0.24	44
nicofibrate	—	-0.02	49	etodolac	U	0.13	53
gemfibrozil	U	0.14	53	phenylbutazone	—	-0.02	49
clofibride	—	-0.10	47	ibuprofen	—	-0.30	43
ciprofibrate	—	-0.08	48	ketoprofen	—	-0.24	44
simfibrate	U	0.28	57	paracetamol	—	-0.42	40
clinofibrate	+	1.97	88	zomepirac	—	-0.32	42
fenofibrate	—	-0.01	49	alprazolam	—	-0.10	48
pravastatin	—	-0.09	48	camazepam	—	-0.26	44
simvastatin	+	0.48	62	chlordiazepoxide	—	-0.17	46
lovastatin	U	0.16	54	phenobarbital	—	-0.17	46
oxiniac acid	—	-0.47	39	ethylsobutyl barb	—	-0.19	45
niceritol	U	0.25	56	butyl butyl barb	—	-0.01	49
nicotinilic acid	—	-0.37	41	cefalotine	—	-0.02	49
nicoclonate	—	-0.21	45	cefazafur	—	-0.04	49
nicotinic acid	—	-0.42	40	cefaclor	—	-0.09	48
pantethine	+	1.10	75	cytarabine	—	-0.40	40
probutol	+	3.81	98	vidarabine	—	-0.40	40
doxazosin	—	-0.09	48	amantadine	U	0.21	55
acifran	—	-0.23	44	uridine ^a	—	-0.47	38
benfluorex	—	-0.24	44	merocianine 540	+	0.47	62
pentaerythritol	—	-0.22	44	guanine ^b	—	-0.40	40
tiadenol	U	0.36	59	pirocatecol	—	-0.42	40
halofenate	—	-0.34	42	pyridine ^c	—	-0.34	42
nafenopin	U	0.16	54	sulfisomidine	—	-0.32	42
pirozadil	—	-0.40	40	sulfadimidine	—	-0.32	42
				sulfamethoxazole	—	-0.33	42
				acebutolol	—	-0.25	44
				atenolol	—	-0.31	42
				bunolol	+	0.46	61
				clenbuterol	U	0.27	57

undetermined (U) = 27.6%, false inactivity = 58.6%,
overall accuracy = 13.8%, adjusted accuracy
(excluded undetermined) = 19.0 %

undetermined (U) = 8.8%, false activity = 5.9%,
overall accuracy = 85.3%, adjusted accuracy
(excluded undetermined) = 93.5 %

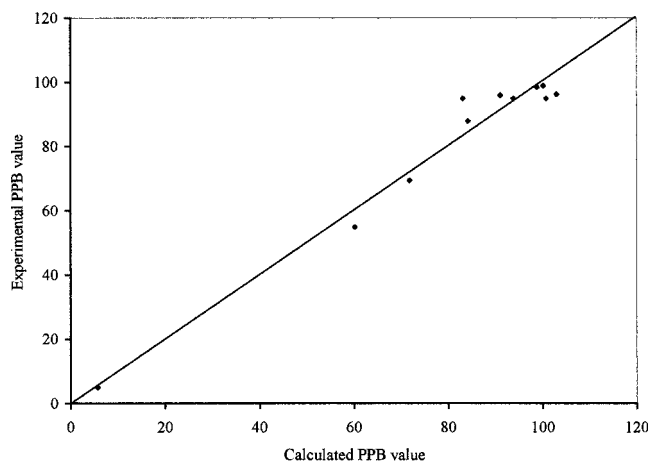
^a 2-deoxy-5-trifluor methyl uridine. ^b 9(1,3-dihydroxy-2-propmethyl) guanine. ^c 2-[2,6-(Iso-C₃H₇O)₂-C₆H₅benzoylamino]-3,5-dichloropyridine.

measurements and in their standard deviation, and this aspect of the study strengthens the predictive quality of the model.

A comparison between the experimental and theoretical values for the selected properties is shown in Figures 2–4, respectively.

In the linear discriminant analysis, the connectivity indices and the pharmacological properties are used as independent variables of each molecule. The best discrimination function was obtained with the variables PPB, T_{\max} , and LDm. Table 7 shows the discriminant function (ΔP), obtained as the difference between the functions defining the groups of active and inactive molecules, together with the values for *F*-Snedecor and Wilk's *U* statistical parameters used with each variable. Molecules with discriminant function values higher than zero ($\Delta P > 0$) were classified as active, while $\Delta P < 0$ corresponds to inactive molecules.

Table 8 shows the results obtained using 29 molecules with demonstrated hypolipaeic activity and 34 inactive molecules in the linear discriminant analysis. Compounds were classified as indeterminate if the probability of activity

**Figure 2.** Correlation between experimental and calculated PPB percentage values of hypolipaeic drugs (equation in Table 3).

or inactivity was between 50% and 60% ($0 < \Delta P < 0.4$); i.e., the margin was too tight for a decision to be made as to whether they were active or inactive. Misclassified com-

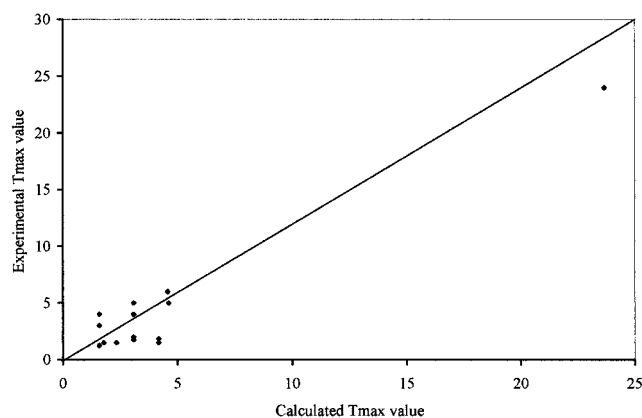


Figure 3. Correlation between experimental and calculated T_{\max} values of hypolipaeic drugs (equation in Table 3).

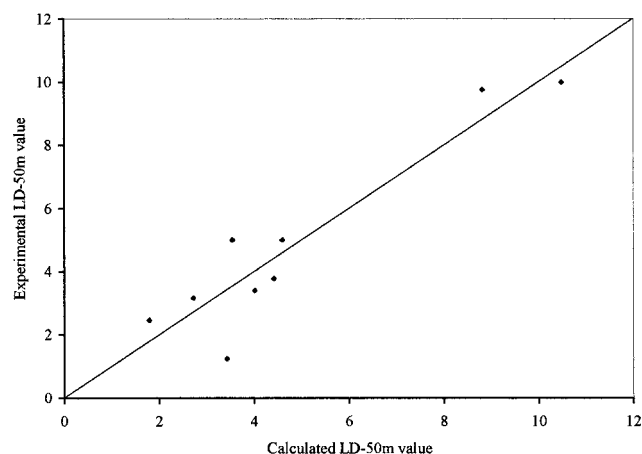


Figure 4. Correlation between experimental and calculated LD-50m (p.o.) values of hypolipaeic drugs (equation in Table 3).

pounds are those whose results were incorrectly predicted by the final LDA equation.

Overall accuracy was 13.8% in the active group and 85.3% in the inactive group. These percentages increase to 19.0% and 93.5%, respectively, if the undetermined molecules are eliminated. The cross-validation test was applied to the ΔP function with a group of 43 hypolipaeic agents and 32 theoretical inactives not used in the discriminant function. Table 9 shows the results obtained. The fact that both the overall accuracy (%) and the adjusted accuracy (%) are similar to those of the group used in the LDA demonstrates the quality of the selected discriminant function.

The equation obtained should, if possible, identify all the non-hypolipaeics, even though the percentage of correct identifications in the active group may decrease. In this context it should be pointed out that, in both the discriminant analysis itself and the validation test, the percentage of false positives is very small (5.9% and 0.0%, respectively), which is what we need to design molecules correctly.

With this procedure we obtained a relatively low total percentage of correct identifications in the group of active molecules (19.0% in the discrimination group and 29.0% in the validation group), but the percentage of correct identifications in the group without hypolipaeic activity was nearly 100% (93.5% in the discrimination group, 100% in the validation group).

Graphing the ΔP value of each molecule in both the active and inactive classifications and both the reference and test

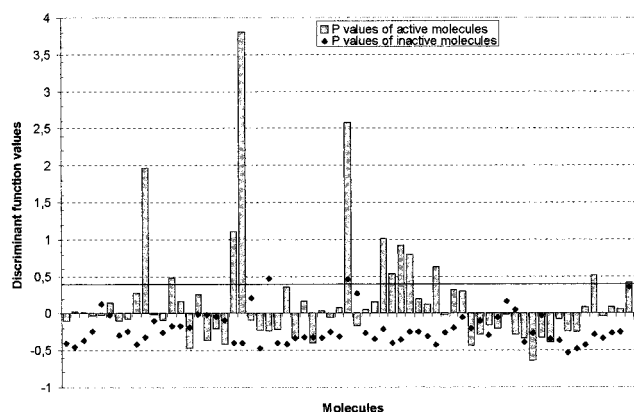


Figure 5. Discriminant function values of active and inactive molecules.

groups (cross-validation) shows that, with the limits mentioned above, only 3% of the inactive molecules are erroneously classified as active (Figure 5).

The discriminant functions are capable of describing pharmacological activity patterns and also nonactivity patterns. In other words, these functions group not only the active drugs according to their distribution but also the inactive compounds. When applied to the discrimination of concrete pharmacological actions, we call them a *pharmacological distribution diagram (PDD)*.²²

A PDD is a frequency distribution diagram of a dependent variable in which the ordinate represents the expectancies of this variable for every interval. The expectancies of this variable are defined as the probability that a compound will be active or inactive for a value of the discriminant function and are obtained by means of the expressions indicated in the text, in which 100 appears in the denominator to avoid dividing by zero:

activity expectancy

$$E_a = \frac{\text{percentage of actives}}{\text{percentage of inactives} + 100}$$

inactivity expectancy

$$E_i = \frac{\text{percentage of inactives}}{\text{percentage of actives} + 100}$$

The main advantage of these diagrams is that they make it possible to determine visually the intervals of the property in which there is a maximum probability of finding new active compounds and a minimum of encountering inactive ones.

Figure 6 shows the classification function obtained through stepwise linear discriminant analysis and the corresponding PDD. In this plot, E_i overlaps the E_a region, but in spite of this the E_i of both the reference and test groups tends toward zero above a discriminating function value of 0.4. After this value, then, we find only active compounds. The values calculated for the discriminant function and the corresponding classification appear in Tables 8 and 9.

To design new active compounds, we used the software package developed in our research unit²¹ and mentioned above. For each molecule designed, the program calculates the corresponding topological indices and pharmacological parameters (with the help of the connectivity functions) and

Table 9. Results Obtained By Applying the Final Discriminant Function to a Group of Compounds Not Included in the LDA (Cross-Validation)

compd	active group classif	ΔP	probability (%)	compd	inactive group classif	ΔP	probability (%)
ronifibrate	U	0.03	51	mefenamic acid	—	-0.27	43
theofibrate	—	-0.06	49	alclofenac	—	-0.35	41
binifibrate	U	0.07	52	azapropazone	—	-0.22	45
etofibrate	+	2.57	100	diflunisal	—	-0.40	40
clofibrilic acid	—	-0.17	46	phenacetin	—	-0.36	41
beclobrate	U	0.05	51	fenoprofen	—	-0.25	44
triparanol	U	0.15	54	indomethacin	—	-0.25	44
β -sitosterol	+	1.01	73	naproxen	—	-0.31	42
mytatrienediol	+	0.53	63	salicylate methyl	—	-0.43	39
furazabol	+	0.92	72	aminoflunitrazep	—	-0.26	43
azacosterol	+	0.80	69	clobazam	—	-0.20	45
clomestron	U	0.19	55	clotiazepam	—	-0.06	49
mevastatin	U	0.12	53	allobarbitol	—	-0.21	45
nicomol	+	0.63	65	prop prop barbit	—	-0.10	47
thyropropic acid	—	-0.02	49	barbital	—	-0.30	43
etiroxate	U	0.31	58	cefradine	—	-0.06	49
melinamide	U	0.29	57	cefazidone	U	0.16	54
eritadenine	—	-0.44	39	cefatrizine	U	0.05	51
α -phenylbutyramide	—	-0.29	43	acyclovir	—	-0.39	40
xenbucin	—	-0.16	46	idoxuridine	—	-0.27	43
sultosilic acid	—	-0.21	45	arildona	—	-0.03	49
eicosapentanoic acid	—	-0.01	49	uridine ^a	—	-0.35	41
meqlutol	—	-0.29	43	guanine	—	-0.37	41
β -benzalbutyramide	—	-0.34	42	metilgalate	—	-0.53	37
metformin	—	-0.64	35	gentisic acid	—	-0.48	38
phenformin	—	-0.33	42	dichloropyridine ^b	—	-0.43	39
buformin	—	-0.39	40	sulfadiazine	—	-0.29	43
acetohehexamide	—	-0.08	48	sulfamethoxypyr	—	-0.34	42
carbutamide	—	-0.24	44	sulfaethoxypyr	—	-0.27	43
chlorpropamide	—	-0.25	44	alprenolol	—	-0.25	44
glibenclamide	U	0.08	52	bufuralol	U	0.36	59
glibornuride	+	0.51	62	bupranolol	U	0.30	57
gliclazide	—	-0.04	49				
glipentide	U	0.08	52				
glipizide	U	0.06	51				
gliquidone	+	0.42	61				
tolazamide	—	-0.09	48				
tolbutamide	—	-0.22	46				
glisoxepid	U	0.02	50				
glybuzole	+	0.45	61				
glymidine	—	-0.25	44				
tolcycyclamide	—	-0.07	48				
metahexamide	—	-0.11	47				

undetermined (U) = 27.9%, false inactivity = 51.2%,
overall accuracy = 20.9%, adjusted accuracy
(excluded undetermined) = 29.0%

undetermined (U) = 12.5%, false activity = 0.0%,
overall accuracy = 87.5%, adjusted accuracy
(excluded undetermined) = 100%

^a 3-Ac-2,3-desoxy-5(methylamino) uridine. ^b 2-[2,6-(CH₃O)₂-1,4-CH₃-C₆H₃benzoylamino] 3,5-dichloropyridine.

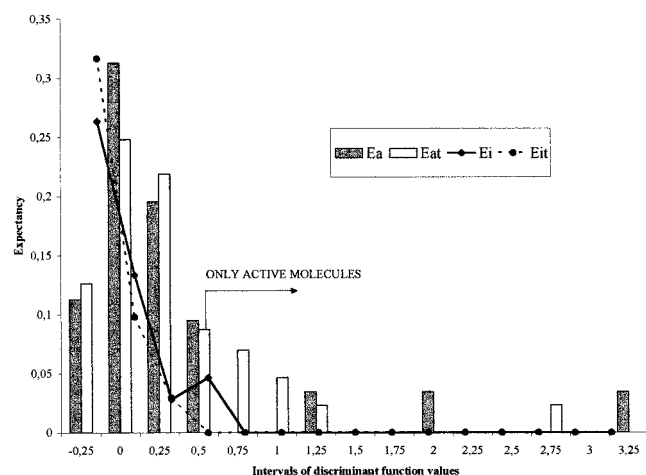


Figure 6. Pharmacological distribution diagram (PDD) for the discriminant function of hypolipaemic activity (E_a and E_{at} , activity expectancy of reference and test groups, respectively; E_i and E_{it} , inactivity expectancy of reference and test groups, respectively). uses them in the discrimination functions for hypolipaemic activity. The molecule designed is selected if it passes the

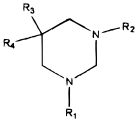
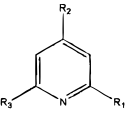
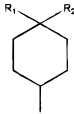
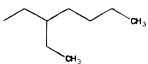
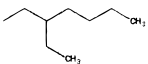
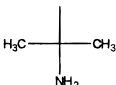
barrier imposed by the discriminant function, in our case $\Delta P > 0.4$ (probability of activity > 60%).

The different base structures used in this design step are collected in Table 10. Structure I yielded pyrimidinic derivatives with substituents at positions 1 (R_1), 3 (R_2), and 5 (R_3 , R_4). Structure II is generic, based on the pyridinic ring with possible substituents at positions 2 (R_1), 4 (R_2), and 6 (R_3). Structure III is a cyclohexane derivative with possible substituents at positions 1 (R_1 , R_2) and 4 (R_3). After computer analysis a total of four compounds with ΔP values > 0.4 were selected as theoretical new hypolipaemic molecules.

CONCLUSIONS

The usefulness of applying molecular topology to identify and design new hypolipaemic drugs is clear from the pharmacological hypolipaemic effect obtained with the group of compounds selected. The discriminant function obtained makes it possible to decrease the percentage of false positives, which is desirable at the molecular design stage.

Table 10. Designed and Selected Chemical Structures

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>I</p> </div> <div style="text-align: center;">  <p>II</p> </div> <div style="text-align: center;">  <p>III</p> </div> </div>						
Compound	Base Structure	R ₁	R ₂	R ₃	R ₄	ΔP
P1 Hexetidine (FW=339.61)	I			- NH ₂	- CH ₃	0.45
P2 2,6-Di-tert-butylpyridine (FW=191.32)	II	- C(CH ₃) ₃	H	- C(CH ₃) ₃	-	0.89
P3 2,6-Di-tert-butyl-4-methylpyridine (FW=205.35)	II	- C(CH ₃) ₃	- CH ₃	- C(CH ₃) ₃	-	0.87
P4 1,8-Diamino-p-menthane (FW=170.30)	III	- CH ₃	- NH ₂		-	0.45

The PDD constitutes a valuable tool in discriminating and consequently in searching for new lead drugs. By means of this function and using the software developed in our research unit, we have obtained new structures with theoretical hypolipaeic activity.

These results verify the method proposed and suggest that it constitutes a simple tool for finding chemical structures which can become new hypolipaeic drugs.

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REFERENCES AND NOTES

- (1) Darvas, F.; Erdos, I.; Teglas, G. *QSAR in Drug Design and Toxicology*; Elsevier: Amsterdam, 1987.
- (2) Pérez Giménez, F.; Antón Fos, G. M.; García March, F.; Salabert Salvador, M. T.; Cercós del Pozo, R. A.; Jaen Oltra, J. Prediction of chromatographic parameters for some anilines by molecular connectivity. *Chromatographia* **1995**, *41* (3/4), 167–174.
- (3) García March, F.; Antón Fos, G. M.; Cercós del Pozo, R. A.; Pérez Giménez, F.; Salabert Salvador, M. T.; Jaen Oltra, J. Correlation of pharmacological properties of a group of beta blocker agents by molecular topology. *J. Pharm. Pharmacol.* **1995**, *47*, 232–236.
- (4) Cercós-del-Pozo, R. A.; Pérez-Giménez, F.; Gálvez-Alvarez, J.; Salabert-Salvador, M. T.; García-March, F. J.; Antón-Fos, G. M. Correlation of pharmacological properties of a group of hypolipaeic drugs by molecular topology. *J. Pharm. Pharmacol.* **1996**, *48*, 241–245.
- (5) Kier, L. B.; Hall, L. H. In Bauden, D. *Molecular Connectivity in Structure-Activity Analysis*; Research Studies Press: Letchworth, England, 1986.
- (6) Gálvez, J.; García-Domenech, R.; Julián-Ortiz, J. V.; Soler, R. Topological approach to drug design. *J. Chem. Inf. Comput. Sci.* **1995**, *35*, 272–284.
- (7) Kier, L. B.; Hall, L. H. *Molecular Connectivity in Chemistry and Drug Research*; Academic Press: London, 1976; pp 46–79.
- (8) Kier, L. B.; Hall, L. H.; Murray, W. J.; Randic, M. Molecular connectivity I: Relationship to non-specific local anesthesia. *J. Pharm. Sci.* **1975**, *64*, 1971–1974.
- (9) Ciudad, J.; García, R.; Gálvez, J.; Algoritmo para el cálculo de los índices de conectividad. Su aplicación a la determinación de la refracción molar de un grupo de moléculas. *An. Quím.* **1987**, *83*, 385–389.
- (10) Dixon, W. J. *BMD Manual: Biomedical Computer Programs*; University of California Press: Berkeley and Los Angeles, 1982; pp 264–277.
- (11) Gray, H. L.; Shucany, W. R. *The generalized Jackknife statistic*; Marcel Dekker: New York, 1972; pp 1–92.
- (12) Marshall, F. N. Pharmacology and toxicology of probucol. *Artery* **1982**, *10*, 7–21.
- (13) Monk, J. P.; Todd, P. A.; Bezafibrate. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hyperlipidaemia. *Drugs* **1987**, *33*, 539–576.
- (14) Vozeh, S.; Schmidlin, O.; Taeschner, W. Pharmacokinetic drug data. *Clin. Pharmacol* **1988**, *15*, 254–282.
- (15) Illingworth, D. R.; Bacon, S. Treatment of heterozygous familial hypercholesterolemia with lipid-lowering drugs. *Arteriosclerosis* **1989**, *9* (suppl 1) 1-121–1-134.
- (16) Alberts, A. W. Lovastatin and simvastatin, inhibitors of HMG-CoA reductase and cholesterol biosynthesis. *Cardiology* **1990**, *77*, 14–21.
- (17) Grundy, S. M.; Vega, G. L.; Garg, A. Use of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in various forms of dyslipidemia. *Am. J. Cardiol.* **1990**, *66*, 31B–38B.
- (18) Hunninghake, M. D. Drugs treatment of dyslipoproteinemia. *Endocrinol. Metab. Clin. North. Am.* **1990**, *9*, 345–360.
- (19) Pan, H. Y. Clinical pharmacology of pravastatin, a selective inhibitor of HMG-CoA reductase. *Eur. J. Clin. Pharmacol.* **1991**, *40*, S15–S18.
- (20) Tilly-Kiesi, M.; Tikkanen, M. J. Low density lipoprotein and composition in hypercholesterolaemic men treated with HMG-CoA reductase inhibitors and Gemfibrozil. *J. Intern. Med.* **1991**, *229*, 427–434.
- (21) Gálvez, J.; García-Domenech, R.; Bernal, J. M.; García-March, F. Desarrollo de un nuevo método de diseño de fármacos por topología molecular. Su aplicación a analgésicos no narcóticos. *An. Real Acad. Farm.* **1991**, *57*, 533–546.
- (22) Gálvez, J.; García-Domenech, R.; de Gregorio Alapont, C.; de Julián-Ortiz, J. V.; Popa, L. Pharmacological distribution diagrams: A tool for the novo drug design. *J. Mol. Graphics* **1996**, *14*, 272–276.